The First Asian Fisheries Forum

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Proceedings of The First Asian Fisheries Forum,
Manila, Philippines, 26-31 May 1986

Edited by

J.L. Maclean
L.B. Dizon
L.V. Hosillos

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EDITED BY

J.L. MACLEAN
L.B. DIZON
L.V. HOSILLOS

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Foreword

The First Asian Fisheries Forum was the first major project of the Asian Fisheries Society, which was founded in 1984. The need for a regular Forum for fisheries scientists in Asia had long been apparent and all 800 respondents to a questionnaire distributed by a Foundation Council for the Society in 1983 affirmed this need.

The theme of this First Forum, "Traditional Practices and New Frontiers in Asian Fisheries" was chosen to highlight the dramatic developments that have occurred in the industry in the last 40 years.

The Forum was hosted by the University of the Philippines in the Visayas (UPV) and the Philippine Bureau of Fisheries and Aquatic Resources. Both organizations contributed a great deal of money and staff time to ensure success of the Forum.

Attendance at the Forum included 289 full participants from 27 countries, 51 students and 68 observers. Their names are given in an Annex to these Proceedings.

Preparations for the Forum began in earnest in November 1984, when a professional congress organizing company, Business Resource Center, Inc. (BRC) was contracted to manage the Forum activities and provide its Secretariat.

A Local Organizing Committee (LOC), headed by UPV Vice-Chancellor Rogelio O. Juliano, was formed to handle the overall management of Forum preparations and activities. Policymaking committees formed under the LOC were the Finance Committee, headed by Chancellor Dionisia Rola; Scientific Program Committee, headed by Dr. Edgardo D. Gomez, and Proceedings Committee, headed by Mr. Jay L. Maclean, who was also Secretary General of the Forum.

An Executive Committee, composed of Prof. Juliano, Dr. Gomez and Mr. Maclean along with BRC, was also formed to take charge of logistics and day-to-day Forum matters.

Fund raising also began in 1984. Efforts of the executive committee which explored overseas sources resulted in the following financial support which is hereby gratefully acknowledged:

- Asian Development Bank, which supported one participant.
- Australian Development Assistance Bureau - International Seminars Support Scheme (ISSS), which provided A$11,000 for partial support for 17 participants.
- The British Council, which funded one participant.
- International Development Research Centre (IDRC) of Canada, which supported 41 participants, as well as CAN$10,000 for Forum preparation and another CAN$10,000 for purchase of Proceedings copies.
- International Foundation for Science (IFS), which supported six participants.
- Royal Norwegian Embassy (India), which funded one participant
- Swedish Agency for Research Cooperation with Developing Countries (SAREC), which provided SEK60,000 (US$9,148.49) for support for six participants.
- United States Agency for International Development (USAID), which funded six participants.

The local finance committee, composed of Chancellor Rola and the late Dr. Elvira Tan, secured the support of University of the Philippines President Edgardo J. Angara, who solicited help amongst government agencies and the industry. Dean Benjamin Catane of the UPV College of Fisheries also wrote to colleagues for donations for the Forum. Dr. Efren Flores made a special trip to Japan to solicit help from colleagues there. As a result of these solicitations, donations by the following organizations and individuals are acknowledged with thanks:

Dakila Trading Ltd.
Reijiro Hirano
Overseas Agro Fisheries Consultants Co., Ltd.
Philippine Commercial International Bank
Philippine Council for Agriculture and Resources Research and Development
Philippine National Bank
Dr. H. R. Rabanal
San Miguel Corporation
Sanritsu Corporation
Southeast Asian Fisheries Development Center (SEAFDEC) Aquaculture Department
Tokyo Sangyo Co. Ltd.
Universal Robina Corp.
West Japan Fluid Engineering

Members of the Scientific Program Committee were: Edgardo Gomez (Chairman) and members were: Virginia Aprieto, Arsenio Camacho, Efren Flores, Jose Llobrera, Alicia Lustre, Inocencio Ronquillo, Alumanda de la Rosa and Leonor Santos.

Local Organizing Committee members were: Rogelio Juliano (Chairman), Arsenio Camacho, Efren Flores, Romeo Fortes, Edgardo Gomez, Jose Llobrera, Jay Maclean, Jose Ordonez, Aurora Reyes, Inocencio Ronquillo and Elvira Tan.

The Forum sessions ran like clockwork thanks to the diligence of all the session chairpersons: Teodoro Abalos, Angel Alcala, Virginia Aprieto, Richard Arthur, Lita Benitez; Arsenio Camacho, Lai Hoi Chaw, Kenji Chiba, Yvonne Chiu, Brian Davy, Sena De Silva, Muhammad Eidman, Pepito Fernandez, Efren Flores, Sonia Formacion, Romeo Fortes, Gloria Guevarra, Reijiro Hirano, Tom Lam, Liao I-Chiu, Aida Librero, Alicia Lustre, John McManus, Jay Maclean, Arnulfo Marasigan, Meng Qing Wen, T.J. Pandian, Twesukdi Piyakarnchana, Roger Pullin, Inocencio Ronquillo, Kenneth Ruddle, Jurgen Saeger, Leonor Santos, Elvira Tan and Carmen Velasquez.

Without the help of all these people and organizations the First Asian Fisheries Forum would not have been the momentous event that indeed took place. There were many other persons, not named here, who lent their time to ensure the smooth operation of the Forum. I am sure that all members of the Society will join with me in thanking all these persons for their efforts.

Finally, those ultimately responsible for the Forum, the First Councillors of the Asian Fisheries Society, are justifiably proud of the fruits of their efforts to begin these triannual conferences, the planning of which began at the birth of the Society almost exactly two years before the First Forum. They were: Chua Thia-Eng (President), William Dall, Brian Davy, Sena De Silva, H.Muhammad Eidman, Edgardo Gomez, Reijiro Hirano (Vice-President), Rogelio Juliano, T.J. Lam, Liao I-Chiu, J. L. Maclean (Secretary from April 1985), Meng Qing Wen, Brian Morton, Richard A. Neal (Secretary 1984 - March 1985), Twesukdi Piyakarnchana, U. Raj, Dionisia Rola (Treasurer) and E.G. Silas.

CHUA THIA-ENG
President
Asian Fisheries Society
December 1986

Preface

The First Asian Fisheries Forum was held in Manila, Philippines, 26-31 May 1986. At the Forum itself, 230 papers were presented. Of these, some were withdrawn for technical reasons or because the authors were publishing them elsewhere, and a more or less equal number was rejected as a result of the review process. The other 167 papers are contained in this book.

To produce these Proceedings in seven months, including correspondence with authors for missing references, original figures, etc., and later the final (computerized) drafts; the review and editing processes; the redrawing of many illustrations, the restyling of most references; production of typeset copy and layout and indexing has been possible only with assistance far beyond the duty of all concerned.

Acknowledgement and sincere appreciation of the efforts of the following are hereby recorded: Eloisa Espiritu and Priscilla Calalang and other staff of the International Center for Living Aquatic Resources Management (ICLARM) who spent many hot weekends (we could not afford the airconditioning) entering and formatting the manuscripts; Julie Dimapilis, who patiently checked and restructured nearly all the references; Mark Anthony Go-Oco, who diligently redrew or modified a good percentage of the figures; the Councilors of the Asian Fisheries Society, especially Dr. Sena De Silva; and the many reviewers who responded in a timely and constructive manner. Special thanks are due to Dr. Ian Smith, Director General of ICLARM, where the Asian Fisheries Society secretariat is located, for allowing and even encouraging staff participation in preparation of the Proceedings.

On the mechanical side, the text of the Proceedings was entered into a microcomputer and typeset through a laser printer. To simplify and speed the layout process, the tables, which were produced separately, and the figures were placed at the end of each paper. We hope authors and readers will appreciate the sacrifice of elegance for speed.

J.L. Maclean
L.B. Dizon
L.V. Hosillos
December 1986

Contents

Foreword v

Preface vii

PLENARY REVIEWS

An overview of the fisheries and aquaculture industries in Asia. CHUA THIA-ENG. **1**

New developments in fish nutrition. AKIO KANAZAWA. **9**

Concepts that work: some advances in tropical fisheries research. DANIEL PAULY. **15**

SESSION PAPERS

Aquaculture Systems

Monoculture

Evaluation of the growth performance of *Oreochromis niloticus* progenies in freshwater ponds. TERESO A. ABELLA, MARIETTA S. PALADA, REMEDIOS B. BOLIVAR and L. JAMES LESTER. **19**

The effects of water depth and circulation on the water quality and production of *Penaeus monodon* in earthen ponds. KENT E. CARPENTER, ARLO W. FAST, VALERIANO L. CORRE, JAMES W. WOESSNER and ROSY L. JANEO. **21**

The prospects of the grass shrimp culture industry in Taiwan. PETER CHIANG, TUOH WANG, KUO CHING-MING and LIAO I-CHIU. **25**

The cycle of nitrogen, carbon and phosphorus in an eel culture pond. KENJI CHIBA. **31**

The use of steel slags as substrates in tilapia *(Oreochromis niloticus)* rearing. CHIEN YEW-HU, LEE CHIN-YU, YAO CHUN-TSU and LAI HONG-TSU. **35**

Evaluation of shrimp *(Penaeus monodon)* hatchery methods practiced by Taiwanese technicians. NORMAN CHWANG LEH-MIN, PETER CHIANG and LIAO I-CHIU. **39**

Possible significance of rearing conditions of ovigerous *Macrobrachium rosenbergii* (De Man). G. GOMEZ DIAZ and A. OHNO. **45**

Production of Nile tilapia fry and fingerlings in earthen ponds at Pila, Laguna, Philippines. RAFAEL D. GUERRERO III. **49**

Cultivation of giant clams: beyond the hatchery. GERALD A. HESLINGA, T.C. WATSON and T. ISAMU. **53**

Settlement and growth of oyster spat in Trincomalee Bay, Sri Lanka. W.M. INDRASENA and T.B. WANNINAYAKE. **59**

The growth rate of milkfish, *Chanos chanos* in brackishwater ponds in the Philippines. ROGELIO O. JULIANO and R. HIRANO. **63**

Mass production of seabass, *Lates calcarifer* (Bloch) by environmental manipulation. PINIJ KUNGVANKIJ and NIWAT SUTHEMECHAIKUL. **67**

Acidification and reclamation of acid sulfate soil fishponds in Thailand. LIN C. KWEI. **71**

The Philippine national *bangus (Chanos chanos)* breeding program. NELSON A. LOPEZ, MA. V.E. VICALDO, M.G. TRIO and J.F. FADRIQUELA. **75**

Effects of teaseed cake on selective elimination of finfish in shrimp ponds. CECILIA LUZ O. MINSALAN and YVONNE N. CHIU. **79**

Water quality dynamics in brackishwater shrimp ponds with artificial aeration and circulation. ROMAN C. SANARES, STEVEN A. KATASE and ARLO W. FAST and K.E. CARPENTER. **83**

Seasonal ponds - a potential source of fish seed in Rajasthan, India. K.P. SHARMA and RAJFISH RANA. **87**

Milkfish pen culture in Sri Lankan lagoons. P.P.G.S.N. SIRIWARDENA. **89**

Intensification techniques in the extensive culture of *Penaeus monodon*. L.B. TIRO, B.J. PUDADERA, I.O. POTESTAS and P. KUNGVANKIJ. **93**

The effect of paddlewheel aerators on ammonia and carbon dioxide removal in intensive pond culture. LEAH MAY B. VER and YVONNE N. CHIU. **97**

Genetics

Triploidy induced by cold shock in cyprinid loach, *Misgurnus anguillicaudatus*. CHAO NAN-HSIEN, CHIEN SHYH-JYI and LIAO I-CHIU. **101**

Diploid gynogenesis induced by cold shock in cyprinid loach, *Misgurnus anguillicaudatus*. CHEN SHYH-JYI, CHAO NAN-HSIEN and LIAO I-CHIU. **105**

Realized response of Thai red tilapia to weight-specific selection for growth. PARNSRI JARIMOPAS. **109**

Hybridization between female red sea bream *(Pagrus major)* and male crimson sea bream *(Evynnis japonica)* by means of sperm cryopreservation. HISASHI KUROKURA, HIDEMI KUMAI and MOTOJI NAKAMURA. **113**

Integrated Farming

Effects of different animal manures on fish farming. FANG YINGXUE, GUO XIANZHEN, WANG JIKUN, FANG XIUZHENG and LIU ZHIYUN. **117**

Effects of fertilizers and feeds as nutrient sources on *Oreochromis niloticus* production in Philippine brackishwater ponds. ROMEO D. FORTES, VALERIANO L. CORRE, JR. and EMELYN PUDADERA. **121**

A preliminary study on sources of fish growth in manured ponds using delta C analysis. GUO XIANZHEN, FANG YINGXUE, WANG JIKUN, FANG XIUZHENG and LIU ZHIYUN. 125

The chemical quality of waste treated waters and its relation with patterns of zooplankton population. K. KAPUR and KULDEEP K. LAL. 129

Relative toxicity of certain livestock wastes for fish culture. K. KAPUR and K.K. LAL. 133

An integrated semi-intensive shrimp and livestock system in the Philippines. DAMIAN M. OGBURN, NEPHERONIA A. JUMALON and MOSES L. SYCIP. 137

Integrated farming of broiler chickens with fish and shrimp in brackishwater ponds. BEATO J. PUDADERA, JR., KAYLIN C. CORRE, ELISEO CONIZA and GIZELLA ANN TALEON. 141

Fecal coliforms as index of pollution in an integrated pig-fish farm system. O.D. QUINES, R.B. VARGAS and V.M. IBARRA. 145

Comparative economics of rice-fish culture and rice monoculture in Ubon Province, northeast Thailand. JOHN D. SOLLOWS and NIRAN TONGPAN. 149

Socioeconomics

An economic analysis of coastal shrimp culture in a mixed farming system, Chittagong-Cox's Bazar region, Bangladesh. A.K.M. MAHFUZUDDIN AHMED. 153

Labor in the dike-pond system of the Zhujiang delta, South China. KENNETH RUDDLE. 157

Biochemistry

Protein moiety of the biliverdin-protein complex in the blood serum of *Clinocottus analis* (Cottidae). FANG LEE-SHING. 161

Polysaccharides of *Gelidium* spp. BETTY MATSUHIRO and CARLOS URZUA. 165

Biology

Developmental

Gill development in the cichlid *Oreochromis niloticus*. CELINE D. DE SILVA and H. THABREW. 169

Morphological development of the swimming and feeding apparatus in larval rabbitfish, *Siganus guttatus*. HIROSHI KOHNO, SHIRO HARA, AMALIA B. GALLEGO, MARIETTA N. DURAY and YASUHIKO TAKI. 173

Morphohistological study of the early development stages of the red sea bream, *Pagrus major*. NELSON A. LOPEZ. 179

Larval rearing of *Macrobrachium rosenbergii* (De Man) in brine solution and sea salt. APOLINARIO V. YAMBOT and EMMANUEL M. VERA CRUZ. **185**

General

Ecology and biology of giant mantis shrimp, *Lysiosquilla maculata* Fabricius in the Philippines. LEA C. ANGSINCO, NATHANIEL C. MENDOZA, WILFREDO H. UY, RODRIGO E. ACUÑA and MANUEL M. TAN. **189**

The offshore environment of Tungkang, Southwest Taiwan (II. Macrobenthos). REN KUWABARA and YUTAKA AKIMOTO. **193**

Elasmobranch fishes in the Nagasaki fishmarket. KAZUHIRO MIZUE and AKIRA TAKEMURA. **199**

Indices of overall growth performance of 100 tilapia (Cichlidae) populations. JACQUES MOREAU, CLAUDIA BAMBINO and DANIEL PAULY. **201**

Distribution and feeding ecology of *Penaeus monodon* along the coast of Tungkang, Taiwan. SU MAO-SEN and LIAO I-CHIU. **207**

The biology and culture of *Ranina ranina* Linnaeus. HELEN J. VICENTE, NATHANIEL C. MENDOZA, ELVIRA O. TAN, RENOIR A. ABREA, RODRIGO E. ACUÑA and WARNITA H. DESTAJO. **211**

Ichthyoplankton studies in the southern Java Sea. N.G. WILLOUGHBY, SUBIYANTO and SOENARYONO PRINGGO SEPUTRO. **215**

Fish Health, Pollution and Toxicity

Bacterial Diseases

Characteristics of gliding bacteria isolated from diseased flounder, *Paralichthys olivaceous*. DOLORES V. BAXA, KENJI KAWAI and RIICHI KUSUDA. **219**

The pathogenicity of bacteria associated with transport-stressed *Chanos chanos* fingerlings. GILDA LIO-PO and ROSELYN DUREMDEZ-FERNANDEZ. **223**

Disease investigation of transported *Chanos chanos* stocked in Laguna Lake, Philippines. GILDA LIO-PO, ROSELYN DUREMDEZ-FERNANDEZ and ANTONIO VILLALUZ. **227**

Aeromonas hydrophila infections in Thailand. KRIENGSAG SAITANU. **231**

Bacteriological examination of fingerlings of bighead carp *(Aristichthys nobilis)* and grass carp *(Ctenopharyngodon idella)* imported into Malaysia. MARIANA NOR SHAMSUDIN. **235**

The susceptibility of various fish species to infection by the bacterium *Aeromonas hydrophila*. HAMBALI SUPRIYADI. **241**

Bacterial flora of seabass, *Lates calcarifer* Bloch, imported from Thailand for cage culture in Penang, Malaysia. WONG SEE YONG and LEONG TAK SENG. **243**

Parasites

Sanguinicola armata infection in bighead carp *(Aristichthys nobilis)* and grass carp *(Ctenopharyngodon idella)* imported into Malaysia. I.G. ANDERSON and FAIZAH SHAHAROM-HARRISON. **247**

Parasite fauna of seabass, *Lates calcarifer* Bloch, from Thailand and from floating cage culture in Penang, Malaysia. LEONG TAK SENG and WONG SEE YONG. **251**

Parasites of Nile tilapia *(Oreochromis niloticus)* in the Philippines. J.M. NATIVIDAD, M.G. BONDAD-REANTASO and J.R. ARTHUR. **255**

Parasites of juvenile milkfish, *Chanos chanos*. S.E. REGIDOR and J.R. ARTHUR. **261**

The reproductive biology of *Dactylogyrus nobilis* (Monogenea: Dactylogyridae) from the gills of bighead carp *(Aristichthys nobilis)*. FAIZAH SHAHAROM-HARRISON. **265**

Identification and distribution of *Lernaea* spp. in Peninsular Malaysia. M. SHARIFF and C. SOMMERVILLE. **269**

The life cycles of *Lernaea polymorpha* and *L. cyprinacea*. M. SHARIFF and C. SOMMERVILLE. **273**

Acquired immunity of *Oreochromis mossambicus* to the ciliate ectoparasite *Ichthyophthirius multifiliis* (Fouquet). ROHANA P. SUBASINGHE and CHRISTINA SOMMERVILLE. **279**

Pollution

Effects of oil on mangrove organisms. LAI HOI CHAW. **285**

Species composition and early development of the benthic marine algae in relation to water pollution in Si Racha Bay, Chonburi, Thailand. HISAO OGAWA and KHANJANAPAJ LEWMANOMONT. **289**

Bacterial depuration of oyster (*Crassostrea iredalei* Faustino) in the Philippines. ELIZABETH QUE PALPAL-LATOC, SUSAN JOCELYN S. CAOILE and ANNABELLE M. CARIAGA. **293**

The effects of sodium cyanide on coral reefs and marine fish in the Philippines. PETER J. RUBEC. **297**

The toxicity of chemical spilt-oil cleaners in Japan. HIROSHI TOKUDA. **303**

Toxicity

Toxic dinoflagellates in Japan. YASUWO FUKUYO and TAKASHI ISHIMARU. **307**

Effects of coconut milk and brown sugar on crude toxins from mussels exposed to *Pyrodinium bahamense* var. *compressa*. ROGELIO Q. GACUTAN. 311

Trace metals in Pacific oysters (*Crassostrea gigas* Thunberg) marketed in Hong Kong. D.J.H. PHILLIPS, E.Y.L. CHAN, E.K.C. PANG and T.L. TING. 315

Toxicity of metal mixtures to *Perna viridis* (Mollusca: Pelecypoda). K.N. PRABHUDEVA and N.R. MENON. 319

Viruses

Isolation of a new rhabdovirus from cultured hirame (Japanese flounder, *Paralichthys olivaceus*) in Japan. T. KIMURA and M. YOSHIMIZU. 323

Snakehead fish virus isolation and pathogenicity studies. KRIENGSAG SAITANU, SOMATAT WONGSAWANG, BOONMEE SUNYASOOTCHAREE and SOMPHONG SAHAPHONG. 327

General Studies

Septicemias of marine crabs and shrimp. CHONG YEN CHUNG and CHAO TIEN MEE. 331

Antibiotic uptake of the giant freshwater prawn *Macrobrachium rosenbergii* by the osmotic infiltration technique. ALCESTIS T. LLOBRERA and DONALD H. LEWIS. 333

Possible cause of mortality of *Oreochromis mossambicus* eggs under artificial incubation. ROHANA P. SUBASINGHE and CHRISTINA SOMMERVILLE. 337

Fisheries

Development and Management

Small-scale fisheries of Iligan Bay, Philippines. RENOIR A. ABREA, NATHANIEL C. MENDOZA, CESARIA S. REYES, LEA C. ANGSINCO, RODRIGO E. ACUÑA and MANUEL M. TAN. 341

Resource management is people management. M. AGUERO N. and B.A. LOCKWOOD. 345

Characteristics of an exploited tropical shallow-water demersal fish community in Malaysia. E.H. CHAN and H.C. LIEW. 349

Trends in reservoir fisheries in Sri Lanka with special reference to *Etroplus suratensis, Tilapia rendalli* and *Oreochromis niloticus*. JAYANTHA CHANDRASOMA. 353

Development of deep sea fishing in the East Sepik province, Papua New Guinea. MOLEAN R. CHAPAU. 357

Effects of the urchin fishery on the population structure of *Anthocidaris crassispina* (Echinodermata: Echinoidea) in Hong Kong. S.T. CHIU. 361

Fisheries development of the Sepik River, Papua New Guinea: proposed fish introductions. DAVID COATES. **367**

Utilization of small-scale fish aggregation devices by Papua New Guinea's artisanal fishermen. S.D. FRUSHER. **371**

The development and management of the Northern Territory barramundi *(Lates calcarifer)* fishery. DARRYL L. GREY. **375**

Spiny lobster fishery in eastern Samar, Philippines. ANTOINETTE R. JUINIO and EDGARDO D. GOMEZ. **381**

An overview of the fisheries of San Miguel Bay, Philippines. ANTONIO N. MINES, IAN R. SMITH and DANIEL PAULY. **385**

Fisheries and fishery management of large reservoirs in Thailand. OOPATHAM PAWAPUTANON. **389**

The fishery of the giant freshwater prawn *Macrobrachium rosenbergii* (De Man) in Sri Lanka. T.B. WANNINAYAKE and H.H. COSTA. **393**

The pot fishery of Lake Biwa, Japan. TAKESHI YAMANE and YUNOSUKE IITAKA. **397**

Education

Fisheries education in China. MENG QING-WEN and WANG TIAN-SHENG. **401**

Progress on the fisheries science applications for microcomputers. CONRAD RECKSIEK, SAUL SAILA and MICHAEL PRAGER. **405**

Gear and Methods

Indigenous fishing crafts in Java, Indonesia. GOZALIE A. GUNAWAN. **409**

Traditional inland fishing methods in Rajasthan, India. S.D. KULSHRESHTHA. **413**

Mesh selectivity and biological impact studies on a new fish-cum-shrimp trawl in Palk Bay, Sri Lanka. M.S.M. SIDDEEK. **417**

The catching mechanism of a prawn pot in Lake Biwa, Japan. TAKESHI YAMANE, YUNOSUKE IITAKA and EFREN FLORES. **421**

Information

Computerized literature searches: their advantages and limitations. BRYAN E. PIERCE. **425**

Postharvest and Marketing

Bacterial depuration of grossly-contaminated oysters, *Crassostrea iredalei*. R.Q. GACUTAN, M.L. BULALACAO and H.L. BARANDA, JR. **429**

Tilapia marketing tests in Kuwait. MARGARITA L. HOPKINS and KEVIN D. HOPKINS. **433**

Effects of storage on the microbial quality of slipper oysters, *Crassostrea iredalei*. ALCESTIS T. LLOBRERA, MINVILU L. BULALACAO and NING SUÑAZ. **437**

The microbiology of cooked rice-minced fish fermentation. L.S. MENDOZA and J.D. OWENS. **443**

The sanitary quality of four Philippine oyster-growing areas. ELIZABETH QUE PALPAL-LATOC, ROBERTO P. GARCIA, SUSAN JOCELYN S. CAOILE, CESAR Z. LUNA, ANNABELLE M. CARIAGA and SAMUEL A. FERNANDEZ. **447**

Household demand for fish in Iloilo City, Philippines. BENEDICT C. POSADAS. **451**

Postharvest spoilage of shrimp *(Penaeus monodon)*. A. REILLY, E. DANGLA and A. DELA CRUZ. **455**

Quality loss and wastage in the processing and preparation of fresh fish in Sri Lanka. S. SUBASINGHE and J.C. GODAKANDA. **459**

Postharvest histamine formation in dolphinfish in southern Taiwan. SUN CHAO-TUNG and PERNG CHANG-YANG. **463**

Improved bycatch utilization in Thailand. SIRILAK SUWANRANGSI. **467**

Effect of raw material freshness on the quality of smoked tilapia *(Oreochromis niloticus)*. L.M. TRINIDAD and M.H. ESTRADA. **471**

Production and storage of smoked Spanish mackerel *(Scomberomorus commerson)*. L.M. TRINIDAD, M.M. NARIDA and A. REILLY. **473**

Resource Assessment

An assessment of the exploitation of coral reef fishery resources in Papua New Guinea. P. DALZELL and A. WRIGHT. **477**

Depth zonation in a demersal fishery in the Samar Sea, Philippines. JOHN W. McMANUS. **483**

Survey of coral reefs, Iligan Bay, Philippines. NATHANIEL C. MENDOZA, WILFREDO H. UY, LEA C. ANGSINCO and MANUEL M. TAN. **487**

Some practical extensions to Beverton and Holt's relative yield-per-recruit model. DANIEL PAULY and MINA L. SORIANO. **491**

Reef fish yields and nonreef catch of Pamilacan Island, Bohol, Philippines. G.C. SAVINA and A.T. WHITE. **497**

Yield-per-recruit analysis of ten demersal fish species from the Samar Sea, Philippines. GERONIMO T. SILVESTRE. **501**

Effects of environmental variability and fishing pressure on the catches of penaeid prawns in the Gulf of Carpentaria, Australia. D.J. STAPLES. **505**

Biology and fishery oceanography of mackerels and scads in the adjacent waters of Taiwan. WANN NIAN TZENG. **511**

Nutrition and Feeding Habits

Aspects of the biology of a tropical cyprinid, *Hampala macroolepidota* (Van Hasselt), with reference to food, feeding habits and reproduction. AIZAM ZAINAL ABIDIN. **515**

Combinations of dietary fat sources in dry diets for *Chanos chanos* fingerlings. VERONICA R. ALAVA. **519**

Azolla pinnata as a dietary component for Nile tilapia, *Oreochromis niloticus*. GAUDIOSA J. ALMAZAN, ROGER S.V. PULLIN, AURORA F. ANGELES, TERESITA A. MANALO, RAMON A. AGBAYANI and MA. THERESA B. TRONO. **523**

Importance of initial laboratory feeding for golden perch fry. PHILIP T. ARUMUGAM. **529**

Growth and survival of Nile tilapia fingerlings in net cages without supplemental feed in Laguna Lake, Philippines. ZUBAIDA BASIAO and ANTONIO SAN ANTONIO. **533**

Formulation and evaluation of pelleted fish feed based on soyabean meal and squilla meal. K.GANAPATHI BHAT, H.P.C. SHETTY and M.C. NANDEESHA. **539**

Effect of amino acid supplementation and vitamin level on the growth and survival of milkfish *(Chanos chanos)* fry. YVONNE N. CHIU, ARSENIO S. CAMACHO and MARY AGNES S. SASTRILLO. **543**

Preliminary studies of factors affecting the feeding rhythm of milkfish (*Chanos chanos* Forskal). YVONNE N. CHIU, MA. PAZ MACAHILIG and MARY AGNES S. SASTRILLO. **547**

Ingestion rate of brine shrimp nauplii by *Metapenaeus ensis*: effects of food concentration and starvation. K.H. CHU and C.K. SHING. **551**

Food, feeding and fecundity of the giant freshwater prawn *Macrobrachium rosenbergii* from natural habitats in Sri Lanka. H.H. COSTA and T.B. WANNINAYAKE. **555**

Optimum ration and feeding frequency in *Oreochromis niloticus* young. SENA S. DE SILVA, RASANTHI M. GUNASEKERA and CHITRA KEEMBIYAHETTY. **559**

Effects of a combination of poultry manure and varying doses of urea on the growth and survival of cultured carps. K.R. DINESH, T.J. VARGHESE and M.C. NANDEESHA. **565**

Diet and feeding chronology of mesopelagic micronektonic fish, *Diaphus suborbitalis*, in Suruga Bay, Japan. YOU-BONG GO. **569**

Feeding habits of larval rabbitfish, *Siganus guttatus* in the laboratory. S. HARA, H. KOHNO, M. DURAY, T. BAGARINAO, A. GALLEGO and Y. TAKI. **573**

The use of freshwater hyacinth *Eichornia crassipes* in cage culture in Lake Rawa Pening, Central Java. JOHANNES HUTABARAT, LACHMUDDIN SYARANI and MICHAEL A.K. SMITH. **577**

Acetes as prime food for *Penaeus monodon* larvae. PINIJ KUNGVANKIJ, A.G. TACON, K. CORRE, B.P. PUDADERA, G. TALEON, E. BORLONGAN and I.O. POTESTAS. **581**

Effect of dietary protein levels on ovarian maintenance and reproductive performance of adult dwarf gourami, *Colisa lalia* (Hamilton). L. LANDESMAN, K.F. SHIM and T.J. LAM. **585**

Suitable diet and optimum feeding frequency in the eyestalk ablated prawn, *Macrobrachium lamarrei*. M. PETER MARIAN, T.J. PANDIAN, S. MATHAVAN, S. MURUGADASS and D.R.D. PREMKUMAR. **589**

The effect of diet on the reproductive performance of pond-reared *Penaeus monodon* Fabricius broodstock. OSENI M. MILLAMENA, JURGENNE H. PRIMAVERA, ROSARIO A. PUDADERA and ROSEMARIE V. CABALLERO. **593**

Enriched conventional feed for Indian major carps. S.N. MOHANTY and D.N. SWAMY. **597**

Stocking density and diet of *Oreochromis niloticus* in cages in manmade lakes in Sri Lanka. II. G. MUTHUKUMARANA and D.E.M. WEERAKOON. **599**

Growth response of four species of carps to different protein sources in pelleted feeds. M.C. NANDEESHA, K.V. DEVARAJ and N.S. SUDHAKARA. **603**

Efficiency of some cyanophytes as larval feed for silver carp *(Hypophthalmichthys molitrix)* and the culture of *Spirulina platensis*. J.B. PANTASTICO, S.F. BALDIA and J.P. BALDIA. **609**

Effect of supplemental lecithin and lipid sources on the growth and survival of *Penaeus monodon* juveniles. FELICITAS PIEDAD-PASCUAL. **615**

Fat-soluble vitamin requirements of the rotifer *Brachionus plicatilis*. CYRIL GLENN SATUITO and KAZUTSUGU HIRAYAMA. **619**

Physiology

Standard and routine metabolic rate, critical oxygen tension and spontaneous scope for activity of tilapias. K. BECKER and LEV FISHELSON. **623**

Metabolic and osmotic responses of *Metapenaeus monoceros* (De Haan) subjected to sudden salinity changes. CHEN I-MING and FANG LEE-SHING. **629**

The effect of salinity on the osmotic and ionic concentrations in the hemolymph of *Penaeus monodon* and *P. penicillatus*. CHENG JIN-HUA and LIAO I-CHIU. **633**

Osmoregulation in *Penaeus monodon*: effects of molting and external salinity. RONALDO P. FERRARIS, FE D. PARADO-ESTEPA, EVELYN G. DE JESUS and JOCELYN M. LADJA. **637**

Effects of dissolved oxygen, temperature and salinity on the oxygen consumption of the grass shrimp, *Penaeus monodon*. LIAO I-CHIU and TADASHI MURAI. **641**

Ontogenetic changes in the composition and energy budget of *Macrobrachium malcolmsonii*. S. MATHAVAN, S. MURUGADASS and M. PETER MARIAN. **647**

Reproduction

Egg size and larval size among teleosts: implications to survival potential. TEODORA BAGARINAO and CHUA THIA-ENG. **651**

Effects of ammonia and nitrite on larval development of the shrimp *(Penaeus monodon)*. CHEN JIANN-CHU, CHIN TZONG-SHEAN and LEE CHIN-KAI. **657**

Induced spawning and larval rearing of grouper (*Epinephelus salmoides* Maxwell). PINIJ KUNGVANKIJ, L.B. TIRO, B.P. PUDADERA and I.O. POTESTAS. **663**

Induction of gonadotropin secretion and ovulation in teleosts using LHRH analogs and catecholaminergic drugs: a review. LIN HAO-REN and R.E. PETER. **667**

Collection of naturally-spawned milkfish eggs in floating cages. CLARISSA L. MARTE, JOEBERT TOLEDO, GERALD QUINITIO and ANTONIO CASTILLO. **671**

Evaluation of a new androgen (Mibolerone) and procedure to induce functional sex reversal in tilapia. FRANK H. MERIWETHER and E.L. TORRANS. **675**

Variations in egg qualities of the Japanese whiting, *Sillago japonica*, in the spawning season. YOSHIOKI OOZEKI and REIJIRO HIRANO. **679**

Induced spawning of *Clarias macrocephalus* (Gunther). THALATHIAH SAIDIN. **683**

Induced spawning of *Pangasius sutchi* (Fowler) using an analog of luteinizing releasing hormone and homoplastic pituitary extract. THALATHIAH SAIDIN and ABAS FAUZI OTHMAN. **687**

Spawning ecology and behavior of the mahseer *Tor putitora* (Hamilton) in the Himalayan waters of Nepal. TEJ KUMAR SHRESHTHA. **689**

Reproductive strategy of *Labeo dussemerii* and implications of hydroelectric and irrigation projects on the Mahaweli Ganga, Sri Lanka. MICHAEL A.K. SMITH and FAUHIMA JIFFRY. **693**

Induced maturation in ablated *Penaeus notialis* and *Penaeus schmitti*. LAIDA RAMOS TRUJILLO and JURGENNE H. PRIMAVERA. **697**

Spawner size and the biological components of the reproduction process in *Penaeus monodon* Fabricius. C.T. VILLEGAS, A. TRIÑO and R. TRAVIÑA. **701**

Induced spawning and larval development of the razor clam *Solen brevis* Gray (Mollusca: Solenidae) in the laboratory. T.M. WONG, T.G. LIM and C.F. WANG. **703**

Gonadotropin and 17β-oestradiol changes during induced spawning and annual reproductive cycle in Wuchang fish *(Megalobrama amblycephala)*. ZHAO WEIXIN, JIANG RENLIANG, HUANG SHIJIAO and ZHOU HONGQI. **707**

INDEXES

Species **711**

Author **720**

Annex

Forum Participants **723**

An Overview of the Fisheries and Aquaculture Industries in Asia*

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Abstract

While fisheries constitute a small part of the gross national product (GNP) in most nations in Asia, their role in national development is of considerable significance in terms of job employment, foreign exchange earnings, food supply and more importantly socioeconomic stability of the rural areas where the majority of Asian populations live. Reviews of current status of the fisheries industries show that Asia still remains a center of fishery and aquaculture activities, contributing 45% of world fish production. Most nearshore fisheries resources in most nations have been fully or overexploited leaving future expansion of the fishing industry to distant and deep water fishing. The fishing industry is currently undergoing structural adjustment in resource allocation and management as a consequence of the imposition of Exclusive Economic Zones. The changes are expected to affect both traditional and offshore or distant-water fisheries in income distribution and socioeconomic status of the fishing communities. The emergence of the aquaculture industry during the last few decades has given new hope for fishery development and new options for depressed nearshore fishermen. Fishery research and extension has not keep pace with the development of the industry, which also suffers from serious lack of qualified and experienced technical personnel to plan, develop and manage the industries. A review of the current fisheries education system in Asia is necessary to ensure development of appropriate manpower for the industry. Fisheries research is essential and must progress towards developing new dimensions for the industry.

Introduction

Fishing and aquaculture industries play a significant role in contributing fish protein to a large Asian population many of whom suffer from chronic malnutrition (Ravenholt 1982); in providing direct employment to fishermen and indirect employment in fishery-related industries; in assisting in the socioeconomic stability of the rural areas and in recent years in assisting developing countries earn foreign exchange through increasing export of high-priced fish commodities. Unlike cereal protein, fish contains essential amino acids such as lysine and thus serves as an efficient supplement to the low-protein, high-carbohydrate diets of developing nations in Asia. Fish contribute a relatively large share of the animal protein intake and account for 33% of the meager animal protein consumed by the average Asian people (RAPA 1985a). In Southeast Asia, the share could be even higher than 50% as the amount of meat protein consumed is relatively low (Kazuo 1984; Florentino et al. 1985).

More than 15 million fishermen and fish farmers working full time and perhaps twice as many working part time rely on nearby waters for their livelihood. A large bulk of the fishing communities are from three major developing nations: India (6.5 million), China (3.1 million) and Indonesia (2.2 million) and most of these are small-scale fishermen and fish farmers (Table 1).

Fishing is an important source of income. It provides for the establishment of other industries like fish processing, refrigeration, cold storage, boat building and net manufacturing as well as other support services. Fishing can also be a generator of economic development (Robinson 1984). Direct employment for fishermen is vital to rural development which generally offers very little alternative employment opportunity compared with that of urban areas. Because of the large number of fishermen and fish farmers in the rural areas, their economic and social conditions are vital to rural stability (George 1981; Ismail et al. 1982; Iwakiri and Neaz 1982).

Asia is a center of fishing and aquaculture activities. Four out of 10 top fish producing countries in the world are from Asia: Japan, China, India and South Korea. They contribute about 80% of total Asian landings. Seven out of 10 top shrimp producing countries are also from this region: India, Indonesia, China, Thailand, Malaysia, Vietnam and Japan (Fig. 1).

The fishing industry has undergone a period of changes in the past 40 years. It was predominantly a traditional fishing industry before World War II. Postwar expansion of the industry was in response to greater demand for fish protein as a result of rapid economic growth especially in the 1960s and 1970s. More and more fishing vessels were mechanized, offshore and distant-water fishing vessels were constructed. Some nations such as Japan, South Korea, Taiwan and Thailand were able to expand their fishing activities from nearshore to offshore and eventually organized distant-water fishing. The catch increased at an accelerated rate but soon levelled off in the 1970s leaving behind problems of overfishing, displaced fishermen and a depressed fishing industry. The declaration of Exclusive Economic Zones (EEZ) in 1983 has caused changes to the fishing industry and certainly

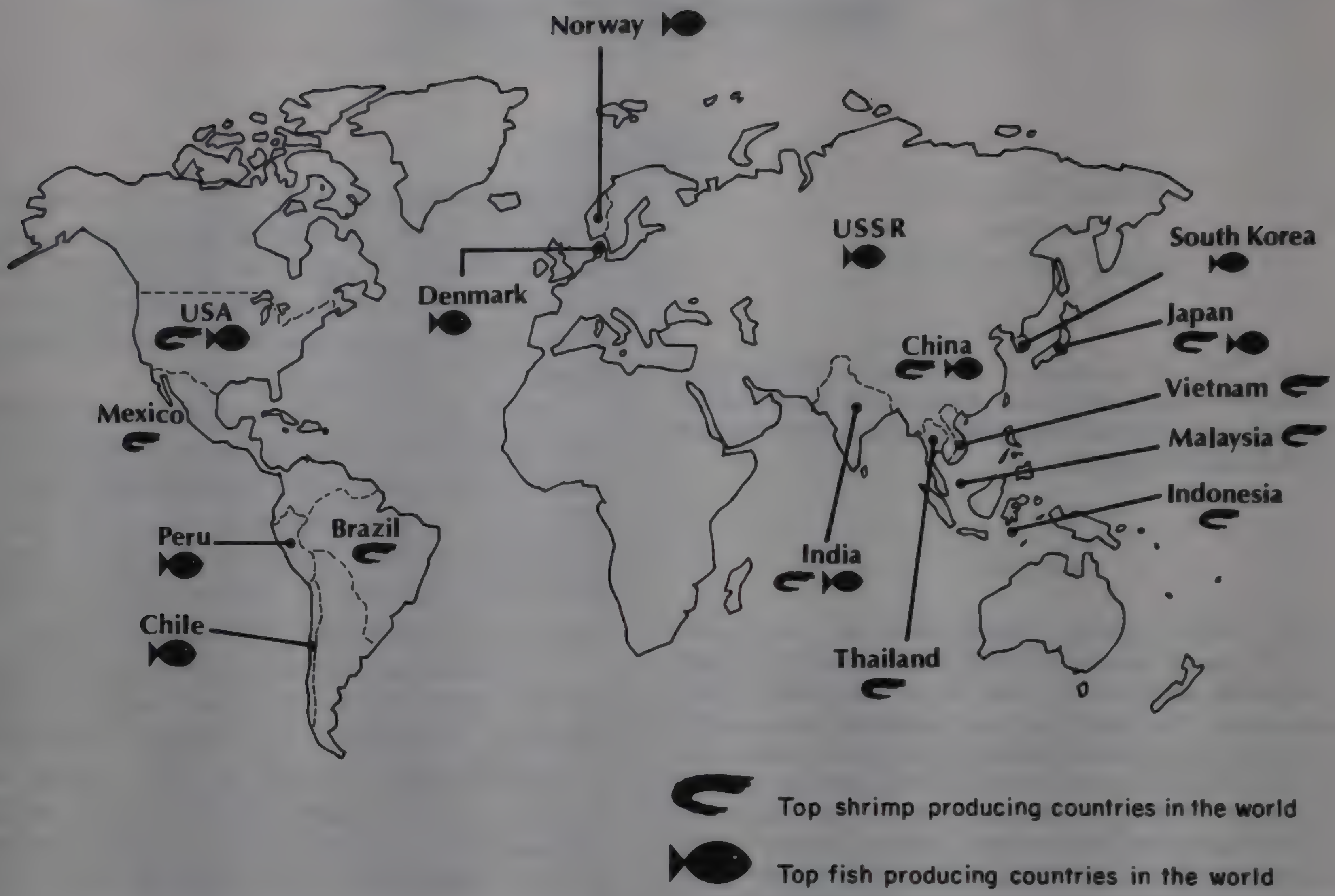

Fig. 1. Top shrimp and fish producing countries in the world.

created considerable impact on the future management of the fisheries resources and the future development of the industry.

Despite the long history of aquaculture in Asia, the industry was relatively underdeveloped. Its potential was realized only in recent years in response to economic and political pressure from many countries looking for alternative measures to increase fish production and employment opportunities for their displaced fishermen. Since the late 1960s, aquaculture has received national and international financial inputs and has expanded into an agro-based industry in many developing nations in Asia.

The current objectives of the fishery sector in the Indo-Pacific region are to increase food supply for domestic consumption and to develop export products for foreign exchange earnings (SEAFDEC/FAO 1985).

Supply and Demand Situation

Asia produced about 45% (35×10^6 t) of world fish landings in 1983 and 65% of the inland fisheries landing. Most of the landings came from East, South and Southeast Asia and less than 1×10^6 t came from west Asia (Near East). Aquaculture contributed 5.24×10^6 t in 1983 (Chua 1986) or approximately 15% of total fish production in Asia.

About 55-60% (ca. 50×10^6 t) of world production are consumed annually. The rest are processed and a substantial amount converted into fish meal as livestock feed. The catches by most developing nations in Asia are mainly used for domestic consumption; only high-priced commodities are exported. In the Philippines, even trash fish are eaten. However, per capita consumption of fish varies from country to country depending on availability, price and sociocultural beliefs. While per capita consumption in Japan (83 kg), Hong Kong (37 kg), Philippines (41 kg), Malaysia (43 kg) and Taiwan (35 kg) are amongst the highest in the world, a number of South Asian nations consume relatively less fish than some of their neighboring countries. Per capita consumption varies from a low of less than 1 kg in Nepal to a high of 7.3 kg in Bangladesh (Table 1). The mean per capita consumption of the region was only 8 kg in 1980 compared to 20 kg in developed nations.

The contribution of fish to direct human consumption could be underestimated as the amounts of fish taken by subsistence fishermen and small-scale fish farmers are not usually reflected in government statistics.

By the year 2000, population growth is expected to be higher in South and Southeast Asia than in developed nations. Another 5×10^6 t will be needed for this region if consumption is to be maintained at the 1980 level

(Robinson 1984). Estimates on the world demand of fish by the turn of the century range from 82 to 104 x 10^6 t (Robinson 1982; Kazuo 1984; Whittle 1985). With the greatest population growth centered in South and Southeast Asia, the pressure for increased fish supply will probably be highest in Asia.

The Fishing Industry

Increasing demand for fish and the lucrative fishing business prior to the 1970s, when most of the offshore fishing grounds were relatively underexploited, have encouraged public and private investment with the development of capital-intensive offshore and distant-water fishing. In order to benefit from the economies of scale, the processing and distribution functions are vertically integrated and the governments usually provide the necessary support facilities in terms of ports, water, electricity, ice factory, cold storage, most of which are part of the fishing port complex usually located in urban areas. In some big companies, factory ships are at sea to process the harvest from distant fishing vessels.

Japan was ahead of the rest of Asia after World War II, spearheading offshore and distant-water fishing, taking advantage of the technical skill of the Japanese fishermen and vast fishery information collected over the past years. With new innovations in equipment and fishing gears, annual fish landing from Japanese vessels quickly reached 10 x 10^6 t in 1972. South Korea and Taiwan, like Japan, ventured into large-scale fishing in offshore and distant waters all over the world. Deep-sea fishing in South Korea has grown fast with exports valued at US$754,000 in 1978 rising to peak at $456.6 million in 1983 (Chen 1984). Some Southeast Asian nations also ventured into offshore fishing but varied in scale of operation. Since the introduction of trawlers in the 1960s, many Southeast Asian nations have rapidly adopted the new fishing technology which initially boosted fish production considerably. The introduction of mechanical seining improved the fishing efficiency of the pelagic fisheries in offshore waters.

The period between the 1950s and 1970s saw the blooming of the fishing industry. Towards the beginning of the 1970s, however, the industry began to encounter serious problems of resource depletion, rising fuel costs and increasing competition among the fishing nations as well as the imposition of the Exclusive Economic Zones (EEZs).

Serious competition between Japanese and South Korean fishing fleets has intensified as both compete heavily for the limited fishery resources in the common offshore waters. Similarly, Taiwanese fishing vessels are increasingly being caught encroaching on Philippine territorial waters while an increasing number of Thai commercial fishermen are being arrested by Burmese and Vietnamese authorities.

Many commercial fishery enterprises in Japan have incurred serious debt through long-term borrowing in order to sustain their current operations. Chen (1984) reports that since the sudden increase in fuel costs in 1979, most fishing enterprises in Japan were forced to take up loans to cover operational expenses and needed capital investment. The industry has since come to rely on loans. Debt-servicing was estimated to be about 240 billion yen in 1983 and outstanding loans more than 4 trillion yen.

The prospect of deep-sea fishing in South Korea is no better than in Japan. The number of fishing vessels has dropped considerably from 878 in 1979 to 646 in 1983. The small companies were soon liquidated while many larger companies are at the brink of bankruptcy.

In general, the commercial fisheries sector of the fishing industry in Asia is facing a murky future. The ability to revive this sector of the industry depends on the ability to enter into more favorable terms in joint ventures with coastal nations which may need infrastructure and technical skill to exploit the offshore renewable resources.

In Asia, small-scale or traditional fisheries still play a dominant role. Traditional fishermen form the bulk of the fishing population, providing 75% of the domestic demand in India, Bangladesh, Burma, Indonesia, Sri Lanka and many other developing nations in Asia. Traditional fisheries are an integral part of rural economy of many coastal nations providing direct employment to millions.

After decades of development, the traditional fishermen in Asia remain in poverty and are among the poorest sector (Devadas 1981; Graham 1982). Even in Malaysia where per capita income is among the highest in Southeast Asia, more than 50% of the traditional fishermen live below the official poverty line, as do 75% of the Philippine population, which includes the fishermen, and a large number in Indonesia, Bangladesh and India. Their fishing boats remain non-powered and very few can afford mechanical gears.

Modern fishing technology has accelerated growth of the fishing industry in terms of production and national revenue but has also caused serious detrimental impact on the fishery resources in many coastal waters. The rapid increase of varying sizes of trawlers indiscriminately reaping valuable fishery resources has resulted in overfishing in most traditional fishing grounds. The Gulf of Thailand, Malacca Straits and coastal waters of the Philippines and Indonesia have been fished to the extent that trawling was banned by the Indonesian government and restricted by many others to reduce fishing intensity and to protect the livelihood of the inshore, traditional fishermen. However, these measures came rather late when most damage had been done.

Overfishing has caused serious reduction in the work force resulting in thousands of traditional fishermen being displaced. About 1/3 of the artisanal fishermen were displaced in Thailand in recent years and those in Malaysia were given alternatives to be resettled in land-based rural agro-industry. The fishermen in Singapore are no better than their counterparts in neighboring countries, being faced with limited marine resources which are already heavily fished. The fishermen are 13% older and earn 20% less on average than the other in the urban state (Chen 1984). There is limited entry of fishermen to the dwindling fishing industry in most Southeast Asian and East Asian nations. Japan faced a similar situation decades back when the younger generation preferred land-based occupations than work at sea.

An exception can be made for the traditional fishermen of the newly independent Brunei Darussalam. Because of the rich oil resources, fisheries resources in the inner Bay of Brunei and the coastal waters on the northwest coast are underexploited and provide livelihood for about 500 fishermen and four times that number of part-time fishermen. Strict immigration control and rigid regulation on entry have enabled the government to keep the fishermen in a manageable number. The estimated annual income (US$3,000) of Brunei fishermen is the highest among fellow inshore fishermen in Asia.

Traditional fishermen are scattered along the coasts, estuaries, rivers, lakes and reservoirs. They are mostly disorganized and many are illiterate. Several attempts to organize them into cooperatives have not met with success in Malaysia and Thailand. On the other hand, fishery cooperatives in Burma and China are active and effective with state support. Many traditional fishermen in Asia depend heavily on financiers or middlemen and most of them are unable to repay their debts.

Aquaculture Industry

Aquaculture in Asia has a history of at least 3,500 years but the industry is still in its infant stage in many Asian countries. The long gestation period in aquaculture development is in part due to the lack of economic pressure and insufficient technical and financial inputs to demonstrate its commercial viability. Aquaculture on a commercial scale has developed into an important food industry in China, Japan, the Philippines and Thailand. It is, however, a relatively new commercial venture in many South Asian nations like Pakistan, Sri Lanka and Bangladesh. Increasing demand for fish supply as a result of population growth, the escalation of oil prices in the 1970s, the growing opportunities in aquaculture industry with respect to employment generation, foreign exchange earning and development of rural economy have created sufficient incentives to develop the industry.

Fish production through aquaculture of 5.24×10^6 t from Asian countries in 1983 (Chua 1986) was lower than the estimates of FAO (RAPA 1985a) of 8.631×10^6 t and Csavas (1985) of 6.206×10^6 t for the same year. China and Japan produced 60% of the Asian total. The developing nations produced 4.1×10^6 t or close to 80% of aquaculture production in Asia. Aquaculture contributes significantly to fish supply in countries such as Nepal (64%) China (36% for mainland, 26% for Taiwan), South Korea (21%) and the Philippines (21%). While aquaculture has helped to raise fish production in Sri Lanka (16%), Malaysia (9%) and Thailand (7%), its contribution to total fish supply in Burma, Pakistan, Hong Kong and India is less than 5% (Chua 1986).

In terms of commodities, Asia leads the world production of seaweeds, finfish, crustaceans and molluscs.

Despite the increase in aquaculture production, most of the fish produced are not within reach of the poorer section of the communities. High-priced commodities such as grouper, eel, yellowtail, seabass, shrimps, are generally limited to the upper class and high production cost for most of these species has greatly limited the expansion of their domestic market. Culture of food fishes low in the food chain entails comparatively less operational cost than raising carnivorous species; however, the production costs of these species are still high and the retail price faces stiff competition with fish of similar quality from capture fisheries.

The market prospects of shrimps are Japan, USA and Western Europe (FAO 1984b) which consume half of the world catch of tropical shrimps. Consumption of shrimp in Japan and USA is not expected to rise significantly for the next few years primarily because of the impact of world economic recession. The European countries may import more than the present quantity and the annual growth is estimated to be 5% (Rackowe 1983). A total of 55,000 t of shrimp (product weight) or 84,000 t (live weight) has been projected as additional imports for Japan, USA and Western Europe by 1990.

The level of aquaculture investment is comparatively lower than that of capture fisheries or other sectors in the agriculture industry. Past investments were confined to the development of small-scale fish farms with very small capital investment, mostly from the fish farmers' own financial resources with little or no public financial support. Investments by the private sector have increased considerably in recent years not only in terms of numbers of new farms established but also in terms of financial magnitude and technological sophistication. In the last few years, many large-scale multimillion aquaculture farms have been developed especially in Japan, China and Southeast Asia adopting vertical integration and central management. However, almost all these fish farms cultivate high-priced commodities with domestic or export potentials such as shrimps, freshwater prawns or crayfish.

Public sector investment has also been increasing due to increased reliance by many countries on aquaculture for their additional fish supply and national policy in rural development. However, public investments either through government inputs or through external aid seem to focus on small-scale aquaculture especially integrated with livestock or crops.

The magnitude of investment in aquaculture is difficult to quantify. Information on investment by private and government sectors is lacking. The level of external aid may reflect present international effort in developing this potential food industry in Asia. Of the total $478.6 million external aid given to fisheries projects in Asia from 1978 to 1982, the proportion allocated for aquacuture was 8.6% in 1978, 11% in 1979 and ranged between 12.2% and 12.8% between 1980 and 1982. Total external aid for aquaculture projects was $88.9 million (1978-1982) of which about 90% were for Asia (Josupeit 1984).

Most of the aid came from the Japanese, the Asian Development Bank (ADB), World Bank, European Economic Community, United Nations agencies and from Western European countries. The World Bank and ADB have been increasing their bank loans for aquaculture development (Loayza 1984).

Despite worldwide interest in the aquafarming industry, large-scale investments in aquaculture projects are still relatively limited. Investors are hesitant in large-scale aquaculture ventures mainly because relatively few aquaculture projects have proven to be a real long-term financial success. Some of the major constraints in enticing private venture in aquaculture, especially in countries where there are no traditional aquaculture practices, are technicians and scientific personnel with hands-on experience in fish farming and farm management; lack of relevant technical and economic information on pilot farms and supply and distribution services such as seeds, feeds and fertilizers (Pillay 1981). The general failure to obtain insurance for aquaculture farms also reflects the instability of the industry.

Management of the Fishing and Aquaculture Industries

The different problems encountered by the fishing and aquaculture industries demand separate management approaches. The depressed situation faced by both traditional and commercial fisheries in most countries in Asia is caused partly by the neglect in management resulting in competitive usage and improper allocation of aquatic fishery resources and partly by the imposition of the territorial fishing rights of most coastal nations. The aquaculture industry, on the other hand, faces problems of economic viability in some countries.

Current issues confronting world fisheries management and development were adequately addressed during the 1984 World Conference of Nations on Fisheries Management (FAO 1984a) during which detailed strategies and action plans for fisheries management and development were adopted.

The main problems faced by unmanaged inshore fisheries in many coastal nations especially Southeast Asia are similar to the constraints outlined by Troadec (1983):

o Limited yield of fishery resources and their frequently high level of exploitation in inshore waters. The situation is aggravated by dynamiting, poisoning and coral reef destruction in many Southeast Asian waters;

o The encroachment on the resource base available to traditional fishermen by large-scale fishing fleets, notably trawlers favored in past national programs;

o The tendency towards overcrowding, notably in countries affected by unemployment, as long as access to local fisheries is not regulated.

Increasing degradation of the coastal environment and inland waters caused by unregulated or insufficient control of the industrial and agricultural activities are additional stress to the already depleted living resources.

Corrective measures depend on government policy and management capability of the coastal nations. Reduction of external pressure on the traditional fisheries through regulation of new entry and prevention of encroachment of offshore fishing vessels are desirable. However, detailed information on the fish stocks and the fishermen is essential to develop guidelines for management policies for traditional fisheries. It is important to stress that fisheries management is not limited to the management of fish stocks but more importantly the people or fishermen that are exploiting them.

Management of commercial fisheries in developing nations is more difficult because of the intrajurisdictional problems which are also faced by Western nations (Regier and Grima 1985). While theoretically it could be regulated through allocation of individual quantitative rights, it faces enforcement difficulties. Management measures recommended include a licensing system to control fishing power of vessels or the quota system by regulating the quantity of catch. The inability of coastal states to exercise effective control and supervision of foreign vessels often results in fishing at will and more often than not, the catch being sent home instead of landing in pre-agreed destinations.

Many public and private aquaculture investments have caused large-scale indiscriminate destruction of valuable mangrove resources for brackishwater pond construction in some Southeast Asian countries. The lack of adequate planning in aquaculture development and absence of management measures to regulate entry often lead to serious social conflicts in resource utilization. These are open management concerns requiring adequate planning and regulation of activities.

Aquaculture is sensitive to aquatic environmental degradation. Inadequate control to prevent pollution can cause serious damage to the industry as experienced in Thailand and Indonesia where disease outbreaks almost wiped out the freshwater aquaculture sector of the aquafarming industry.

Fisheries Research and Extension

Past fisheries research did not keep pace with development of the industry. In most cases, the industry was left on its own and developed through painful experience of failures. Fisheries scientists have not been able to respond adequately to the immediate needs of the industry to provide the necessary information on resource base, appropriate technology, the economics of fishing and aquaculture, and the socioeconomic conditions of fishermen and fish farmers.

With the expansion of territorial fishing jurisdiction to 200 nautical miles from the coast, coastal nations are entrusted with greater responsibility to provide the needed information on fish stocks and measures in managing them.

Over the last 70 years, advances in aquaculture through research have led to significant improvement of fish farming techniques especially in areas of seed production, feeds and yield. The application of Human Chorionic Gonadotropin (HCG) and pituitary extracts to induce maturation and spawning of many species of carp, catfish, yellowtail, seabass and siganids, as well as eyestalk ablation of shrimps, have led to the establishment of hatcheries for mass production of fish seed. High-protein pellet feeds with efficient food conversion have improved yields and promoted automation in fish farming operations. Improvement of engineering design of culture facilities and techniques in grow-out operation have increased yields and production. The increasing use of hatchery-bred fry for stocking is an indication of confidence of fish farmers in the quality of seeds supplied through artificial propagation. Almost 90% of Chinese carps cultured in China are produced from hatcheries while the farming of the freshwater prawn *(Macrobrachium)* is completely dependent on hatchery-bred juveniles.

The advancement from extensive to semi-intensive and then to intensive farming operations using artificial feeds and employing scientific management skill to increase yield in many developing countries reflects the increasing application of scientific research results in aquaculture.

The trend of aquaculture research has changed in recent years to a interdisciplinary oriented approach in order to develop the appropriate technology for the industry. In spite of recent advances in aquaculture research, there are certain problems that need concerted scientific effort for solutions. The industry is still seriously lacking easily available cheap but effective formulated feeds for finfish and especially for shrimp. High-priced artificial feeds may increase production cost and reduce profitability. Adaptive research should also be conducted in the production of artificial feeds using local ingredients and increasing use of plant protein instead of total reliance on animal protein. Research work on various grow-out operations needs to be intensified to improve existing knowledge of optimal carrying capacity of culture facilities and water management. Research effort on selective breeding is also important to maintain constant supply of quality seeds in the future.

Extensive biological research contributed significantly to successful ranching of a number of commercially important species, including red seabream, penaeid shrimp and abalone; mass cultivation of seaweeds; large-scale seeding of inland water bodies (lakes, reservoirs and canals) with hatchery-bred fry or fingerlings; and enhancement of natural habitats through artificial reefs (Alevizon et al. 1985; Bohnsack and Sutherland 1985; Burchmore et al. 1985; Nakamura 1985; Spanier et al. 1985).

Biotechnical research has been the recent trend in developed nations and many such research findings have significance to aquaculture development in Asia. New genetic hybrids (Refstie and Gjedrem 1975; Wilkins 1981; Kinghorn 1983) and success in the technique of cloning will one day enable fish farmers to produce the desirable commodity at will (Sylvester and Waaland 1983). Application of operations research and system design in aquaculture (Orth 1980) has proven of increasing necessity in profit-oriented enterprises.

Another area that requires immediate attention is the extension services to support public fisheries development through creation of public awareness, transfer of appropriate technologies, provision of technical advice and development of support services such as port facilities, ice factories, hatcheries, processing plants, credit and loans.

The major problems encountered by the industry are the serious lack of adequate qualified extension workers and the financial means to develop the necessary support facilities needed for the development and expansion of the fishing and aquaculture industries.

Manpower, Infrastructure and Financing

Most developing nations in Asia face a shortage of qualified manpower to plan, develop and manage the fishing industry which is adjusting to the changes imposed on it as a consequence of EEZs. In the aquaculture industry, trained technical personnel are greatly needed to implement public and private aquaculture development programs. Both aquaculture managers and technicians with practical experience are in great demand and existing training institutions have not been able to supply the needed products.

The number of fishery research scientists working in Asia has yet to be determined. Initial estimates show that about 15,000-20,000 fisheries scientific and technical personnel are working in various developing nations in Asia (Chua, unpublished data) and most of them are biologists. Many do not have formal fishery or aquaculture training. Highly qualified technical personnel with doctorate and masteral degrees are limited in most countries except in most Indian research institutions.

Fishery educational institutions are limited in the region except in the Philippines, where there are exceedingly large numbers (138) of fishery high schools and colleges (Bicol University 1982), much more than the 17 fisheries colleges and 52 fisheries high schools in Japan. In most developing nations, fisheries are incorporated as part of applied science. In many countries, college students take up fisheries as a last choice. This is certainly not a healthy situation in the development of fishery manpower in the region.

While there are adequate funds available in the private sector for fishing and aquaculture investment in some countries, there is serious lack of public funding for planning inputs and infrastructure development. Inputs from governments and through external aid are needed.

Conclusion

The Asian fishing industry is currently undergoing structural adjustment in resource allocation and management as a consequence of the imposition of the EEZs. The changes affect both traditional and offshore or distant-water fisheries in income distribution and socioeconomic status of the fishing communities.

While the fishing industry continues to face an unpredictable future, the emergence of aquaculture poses new hope for fishery development and new options for the depressed fishermen. Fisheries science must progress; the positive impact of scientific research must be felt if fishery scientists are to play an active role in directing research efforts toward the achievement of the common objectives of the fishing and aquaculture industries.

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Table 1. Relative importance of fisheries in Asian nations.

Country	Population (million) (1984)	Employment (fishermen and fishfarmers)	Per capita consumption (kg/person/yr)	DGP/GNP (%)
Bangladesh	99.6	–	7.3	6.2 (GNP)
Burma	38.9	80,000	17.8	–
Brunei	0.22	528	35.5	0.12 (DGP)
China (excluding Taiwan)	1,034.5	3,000,000	4.5	–
China (Taiwan)	19.2	313,000	36	–
Hong Kong	5.4	28,000	37	–
Indonesia	162.2	2,232,000	13.1	1.7 (DGP)
India	746.4	6,500,000	3.0[b]	–
Japan	119.9	460,000	83.0[b]	–
Korea (south)	42	738,000	38.4[b]	1.5 (GNP)
Malaysia	15.3	115,000	43	–
Nepal	16.6	–	0.3	–
Pakistan	97.3	205,000	1.7[b]	0.3 (DGP)
Philippines	54.5	827,000	41[a]	4.5 (GNP)
Singapore	2.5	2,800	31.8	ng
Sri Lanka	16.1	70,000	14	3 (DGP)
Thailand	51.7	500,000	18.8[b]	1.6 (GNP)

[a] Florentino et al. 1985.

[b] RAPA 1985b.

New Developments in Fish Nutrition

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Abstract

More than 300 species of fish have been cultured in various countries as an animal protein source. In aquaculture, although research on fish feeds is important to increase the productivity of fish, detailed information of fish feeds and nutrition is limited in some species. Recently, there have been new developments in fish nutrition that have helped to establish the mass production of finfish and shellfish. This paper presents four of these new developments in fish nutrition.

Microparticulate Diets for Larval Fish

In the seed production of aquatic animals for aquaculture, live foods such as the rotifer, *Brachionus plicatilis* and *Artemia salina* have been widely used throughout the world. However, they require much equipment, maintenance expense and labor to produce a desired amount of live food safely and constantly. Therefore, it is necessary to develop artificial diets as a substitute for live foods to further increase the production of seed for fish culture. We have attempted to rear larval fish with microparticulate diets for several years.

Various types of microparticulate diets have been prepared (Adron et al. 1974; Gatesoupe and Luquet 1977; Gatesoupe et al. 1977; Metailler et al. 1979; Teshima et al. 1982; Kanazawa et al. 1982; Kanazawa and Teshima 1983; Dabrowski et al. 1983; Bromley and Howell 1983; Dabrowski 1984; Charlon and Bergot 1984; Fuze et al. 1985; Kanazawa 1985a, 1985b; Kanazawa et al. 1985a).

The microparticulate diets reported are categorized into three groups as shown below. Microencapsulated diets (MED) are defined as microparticulate diets made by encapsulating a solution, colloid or suspension of diet ingredients with a membrane. Microbound diets (MBD) are powdered diets with a binder. Microcoated diets (MCD) are prepared by coating MBD with some materials such as zein and cholesterol-lecithin.

Microencapsulated diets (MED)
- Nylon-protein MED
- Gelatin-gum acacia MED
- Egg albumin MED
- Glycopeptide MED
- Chitosan MED

Microbound diets (MBD)
- Carrageenan MBD
- Agar MBD
- Zein MBD
- Alginic acid MBD
- Gelatin MBD

Microcoated diets (MCD)
- Zein MCD
- Cholesterol-lecithin MCD
- Nylon-protein MCD

The microencapsulation of diet ingredients with a nylon-protein membrane was fundamentally based on the interfacial polymerization procedures of Chang et al. (1966) and Jones et al. (1979). Fig. 1 shows the outline of preparation of nylon-protein MED.

The details of the procedures for preparation of other microparticulate diets are described elsewhere (Teshima et al. 1982; Kanazawa and Teshima 1983; Kanazawa 1985).

Mass production of ayu and carp seed with microparticulate diets has been accomplished. For ayu *(Plecoglossus altivelis)*, 200,000 larvae were placed in 50-t tank. Feeding trials were carried out for 90 days at 14.5-20.3°C. The larvae received the MBD 10 times daily. The composition of the MBD is shown in Table 1. The dietary values of diets were evaluated in terms of total length, body weight gain, and survival rate. Furthermore, the incidence of malformation was checked 90 days after hatching. Two groups of larval ayu were fed the diets as shown in Fig. 2. One group was mainly live food such as rotifer and artemia, and another group was mainly MBD containing 50% rotifer.

The results of growth and survival of larval ayu reared for 90 days are shown in Fig. 3. The survival of the MBD group at the end of the feeding trial was 88.5%. The total length and body weight of MBD group and live food group were similar. These results indicate that the microparticulate diet has a nutritive value corresponding to the live food in terms of growth and survival rates. Also, the incidence of nutritional deficiency such as scoliosis was rarely found when the ayu larvae were reared with MBD containing lecithin (Kanazawa et al. 1984).

Recently, production of carp seed, *Gnathopogon elongatus caerulescens*, was carried out with microparticulate diet alone. The results gave high growth and survival rates as shown in Fig. 4 (Awano et al. 1986).

Generally, dietary proteins having an essential amino acid (EAA) pattern similar to that of whole body or egg proteins are likely to have a high nutritive value for fish. Therefore, we analyzed the amino acid composition of whole body proteins of ayu, and then made test diets using various protein sources to simulate the amino acid patterns of the body protein of larval ayu. Six microparticulate diets were formulated with white fish meal, brown fish meal, bonito powder, short-necked clam extract powder, feather meal, yeast powder, soybean meal, gluten meal, and/or krill meal (Tables 2 and 3). Their nutritive values were compared with a live food control by a feeding trial for 63 days from 11 days after hatching.

Table 4 shows the results of the 63-day feeding trial. The test diet groups gave 35.3-91.6% survival and 39.53-45.25 mm total length, whereas the live food control receiving the rotifer and artemia afforded 82.8% survival and 45.22 mm total length (Kanazawa et al. 1985b).

Broodstock Diets for Fish Production

It is likely that the nutritional quality of broodstock diets may affect the ovarian maturation, spawning, and egg quality. Little information is available on this field. Watanabe et al. (1984a, 1984b) reported the effects of proteins, lipids, minerals, vitamins and pigments in diets on the quantity and quality of eggs.

For proteins, Watanabe et al. (1984a) examined the effects of feeding broodstock of red sea bream, *Chrysophrys major*, on egg quality, and revealed that the group fed a low protein (crude protein, 36%) diet gave few floating eggs and normal larvae (4% of hatched larvae) as compared with the group fed the control diet (51% protein); in the latter group 62% of the hatched larvae were normal in appearance.

In the case of lipids, Watanabe et al. (1984b) studied the effects of diets on spawning by rearing female rainbow trout *Salmo gairdneri*, (2 years old) with an EFA-deficient diet for three months. As a result, the group fed the EFA-deficient diet afforded low values for the number of eggs produced, the rate of eyed eggs, and hatching rate; these values were improved by the addition of linoleic acid ($18{:}2\,\omega 6$). Watanabe et al. (1984a) also revealed by a 6-month feeding trial of the parent red sea bream that the EFA-deficient diets gave few buoyant eggs and more demersal eggs (about 75% or more). The feeding of corn oil as a substitute of squid liver oil in a basal diet resulted in a marked deterioration of egg qualities and gave few normal fish (1.2% for corn oil diet; 53% for the basal diet).

Recently, Hara (pers. comm.) has shown that the tropical fish *Siganus guttatus* laid eggs for more than five months when receiving a diet with pollack liver oil (PLO) but only for two months when receiving diets without PLO. The data suggest that the inclusion of highly unsaturated fatty acids in diets is necessary for normal reproduction of *S. guttatus*.

Generally, fish eggs contain a large amount of phospholipids. Kanazawa et al. (1981, 1983a, 1983b) demonstrated by feeding trials that the larval ayu, red sea bream, and *Oplegnathus fasciatus* (knife jaw) require dietary sources of phospholipids for their growth and survival. Some information also suggests that dietary sources of phospholipids have certain effects not only on larvae but also on parent fish. Watanabe et al. (1984a) have shown that the phospholipid and astaxanthin fractions of krill meal were effective in improving the quality of eggs.

Watanabe et al. (1984b) have shown that the parent ayu fed the phosphorus-deficient diet had a poor growth and produced fewer eggs. Long-term feeding trials of rainbow trout revealed that the elimination of trace metals resulted in unusual spawning and inferior egg quality besides poor growth and low feed conversion efficiency. In the case of the red sea bream (Watanabe et al. 1984a), the production rate of buoyant eggs was lowered when broodstock received the phosphorus-deficient diet.

Vitamin E is the most important among the essential nutrients in relation to the development of reproductive organs, and it plays a role in spawning and egg qualities as also observed in higher animals. Takeuchi et al. (1981) reared parent ayu with diets containing varying levels of vitamin E for three months before spawning and estimated that the fish require 3.4 mg of vitamin E in 100 g of diet in terms of hatching rate and the survival of hatched larvae. As for the carp, *Cyprinus carpio*, 17-month feeding trials have shown that vitamin-E deficiency in diets resulted in the retardation of ovarian development (Watanabe and Takashima 1977).

Good quality eggs of the red sea bream have been obtained when the parent fish were fed diets containing β-carotene + canthaxanthin, krill oil, or frozen krill supplements for two months before spawning (Watanabe et al. 1984d) (Table 5). Recently, Watanabe et al. (1985) demonstrated that feeding red sea bream broodstock with frozen raw krill after they had previously been fed on a diet fortified with vitamin E for 26 days also resulted in elevation of the percentage of buoyant eggs.

Biochemical Techniques for Evaluating Nutritional Status of Fish

The vitamin requirements of fry and fingerlings are well known. However, adult fish will not show deficiency signs as quickly as fry or fingerlings. Detection of vitamin deficiency in adult fish requires sensitive analytical techniques. Two types of biochemical technique are used in evaluating vitamin nutritional status. One technique is the measurement of vitamin-dependent enzyme activity in the tissue. The other is the measurement of vitamin level in tissue.

We have suggested to compare the estimation of thiamine or pyridoxine status by direct measurement of vitamins in tissue using high-performance lipid chromatography (HPLC) and by measuring the vitamin-dependent enzyme activity from tissue of rainbow trout fed a thiamine-deficient diet, pyridoxone-deficient diet, or complete diet (Hardy et al. 1986; Matsumoto et al. 1986) (Table 6). Rainbow trout fed the pyridoxine-deficient diet exhibited signs of pyridoxine deficiency between 11 and 14 weeks of feeding. However, the degree of pyridoxal phosphate stimulation (% stimulation of aspartate aminotransferase activity) of plasma became significantly different between the pyridoxine-deficient diet and complete diet after four weeks of feeding (Fig. 5). Also, the average body weights of rainbow trout were significantly different between the thiamine-deficient diet and complete diet at 30 weeks of feeding. However, at 16 weeks and thereafter, the thiamine pyrophosphate level of liver was significantly lower in fish fed the thiamine-deficient diet compared to those fed the complete diet (Fig. 6).

From these results, measurement of vitamin coenzyme form is a more important indicator of vitamin status than measurement of vitamin "parent" form. This sensitive clinical test for fish, particularly adult fish, can be used to judge whether the supplemental level of vitamin is suitable or not.

Production of Salmon Growth Hormone by Gene (DNA) Recombination Technology

Goodman and co-workers at the University of California first succeeded in producing growth hormones by a gene recombination technology. By using a genetically engineered *Escherichia coli*, they produced a human-growth hormone in 1979 (Martial et al. 1979). The biotechnological approach to a fish-growth hormone is also interesting in the field of aquaculture.

In Japan, Kawauchi at Kitasato University has isolated and identified a growth hormone from a chum salmon, *Oncorhynchus keta*, (Kawauchi et al. 1983). From 1983 he and the members of Kyowa-Hakko Kogyo Company in collaboration have succeeded in the large-scale production of the chum salmon growth hormone (sGH) by the gene recombination technique using *E. coli*, after the isolation of the gene concerned with the growth-hormone production (Sekime et al. 1985). Now it is possible to produce the sGH which corresponds to 15-40% of whole cell protein of *E. coli* at a rate of 20-30 g/l of incubation media.

The partially purified sGH sample from *E. coli* was used in growth-promoting experiments of rainbow trout (Fig. 7). The results clearly indicate that sGH synthesized in *E. coli* is equipotent to the natural sGH in promoting increases in weight and length of rainbow trout. Fig. 7 shows the results of one injection of 1 μg of growth hormone into the abdominal cavity to the rainbow trout (10 g in body weight, 7 cm in body length). The growth hormone has been shown also to exert the same effect by a dipping method. Further investigtion is going on for practical application to aquaculture.

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Table 1. Composition of microparticulate diet for fish larvae.

Ingredient	g/100 g dry diet
Chicken egg yolk	24.0
Short-necked clam powder	8.0
Bonito extract powder	5.0
Milk casein	10.0
Egg albumin	15.0
Soluble yeast powder	10.0
Amino acid mixture	5.0
Vitamin mixture	8.0
Mineral mixture	6.0
Bonito egg lecithin	3.0
Squid liver oil	4.0
Soybean oil	2.0
Total	100.0

Table 2. Composition of microparticulate diet for ayu.

Ingredient	Diet No. 1	No. 2	No. 3 (g/100 g dry diet)	No. 4	No. 5	No. 6
Feather meal	9.6					
Bonito powder	9.6	19.3	9.6	9.6	39.4	9.6
Krill meal	41.6	31.0	31.0	10.4		31.0
White fish meal	19.2			48.1		
Yeast powder		28.9	19.1			19.1
Gluten meal			20.3		20.3	
Short-necked clam extract powder				10.4		
Brown fish meal					20.0	
Soybean meal						12.5
L-Methionine					0.25	
Cellulose	0.0	0.8	0.0	1.5	0.05	7.8
Squid liver oil	6.0	6.0	6.0	6.0	6.0	6.0
Soybean lecithin	3.0	3.0	3.0	3.0	3.0	3.0
Vitamin mixture	6.0	6.0	6.0	6.0	6.0	6.0
Mineral mixture	5.0	5.0	5.0	5.0	5.0	5.0
Total	100.0	100.0	100.0	100.0	100.0	100.0

Table 3. Ratio of essential amino acids (EAA) relative to methionine in ayu and diet.

EAA	Ayu	Diet No. 1	No. 2	No. 3	No. 4	No. 5	No. 6
Met	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Thr	1.18	1.19	1.24	1.27	1.16	1.15	1.08
Val	1.46	1.46	1.55	1.64	1.52	1.43	1.55
Ile	1.34	1.46	1.47	1.65	1.45	1.25	1.52
Leu	2.39	2.21	2.30	2.39	2.65	2.30	2.73
Phe	1.36	1.73	1.66	2.11	1.94	1.39	1.79
His	0.71	0.72	0.71	0.82	0.78	0.90	0.88
Lys	1.35	1.86	1.98	2.19	1.96	2.10	1.54
Trp	0.89	0.99	0.97	1.23	0.84	1.15	1.18
Arg	2.08	1.93	2.01	2.10	1.88	2.03	1.95

Table 4. Effect of different dietary protein on growth and survival of ayu larvae.

Diet	Initial No. of fish	Total length (mm)	Final Total length (mm)	Body weight (mg)	Survival rate (%)
Microparticulate diet:					
No. 1	2,500	9.19 ± 0.52	44.85 ± 2.66	0.27	89.0
No. 2	2,500	9.19 ± 0.52	45.25 ± 3.42	0.28	84.8
No. 3	2,500	9.19 ± 0.52	41.22 ± 3.33	0.19	91.6
No. 4	2,500	9.19 ± 0.52	44.14 ± 3.06	0.22	82.0
No. 5	2,500	9.19 ± 0.52	39.53 ± 3.82	0.15	35.3
No. 6	2,500	9.19 ± 0.52	41.28 ± 3.71	0.21	88.4
Control:					
Live food	2,500	9.19 ± 0.52	45.22 ± 4.12	0.31	82.8

Table 5. Effect of broodstock diets on the spawning and egg quality of red sea bream.

	Control (High protein)	High protein + β-carotene + canthaxanthin	Protein + krill oil extract	Frozen krill
Egg:				
Eggs produced/fish (x 10^{-4})	149.5	120.4	90.1	202.1
Buoyant egg (%)	49.1	56.4	69.6	82.7
Abnormal egg (%)	77.5	37.0	20.9	8.1
Av. no. of oil globules	2.5	1.8	1.2	1.1
Hatched larvae:				
Hatching rate in buoyant egg (%)	83.1	77.4	67.5	90.3
Deformity (%)	14.8	15.0	8.4	2.0
Normal larvae obtained from buoyant egg	51.6	74.8	88.2	91.2
Rate of normal larvae obtained (%)	21.1	39.1	41.4	68.1

Table 6. Direct and indirect methods for measuring thiamine and vitamin B_6.

	B_1	B_6
	Thiamine	Pyridoxine Pyridoxal Pyridoxamine
Coenzyme	Thiamine pyrophosphate	Phridoxal phosphate Pyridoxamine phosphate
Direct method	Thiamine pyrophosphate	Pyridoxine Pyridoxal
Indirect method	Transketolase	Aspartate aminotransferase (GOT)

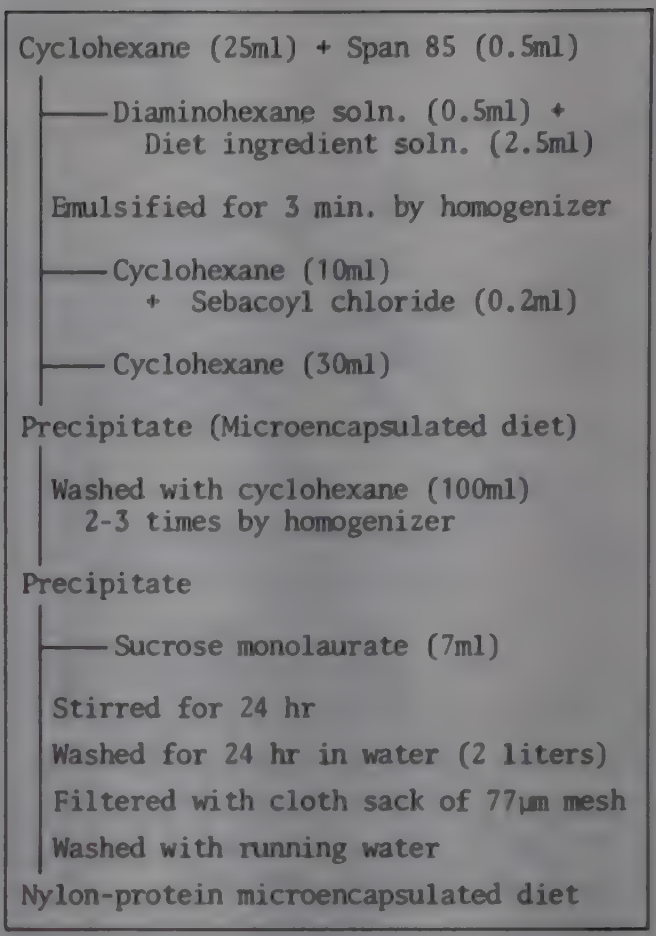
Cyclohexane (25ml) + Span 85 (0.5ml)
Diaminohexane soln. (0.5ml) + Diet ingredient soln. (2.5ml)
Emulsified for 3 min. by homogenizer
Cyclohexane (10ml) + Sebacoyl chloride (0.2ml)
Cyclohexane (30ml)
Precipitate (Microencapsulated diet)
Washed with cyclohexane (100ml) 2-3 times by homogenizer
Precipitate
Sucrose monolaurate (7ml)
Stirred for 24 hr
Washed for 24 hr in water (2 liters)
Filtered with cloth sack of 77μm mesh
Washed with running water
Nylon-protein microencapsulated diet

Fig. 1. Preparation of nylon-protein MED.

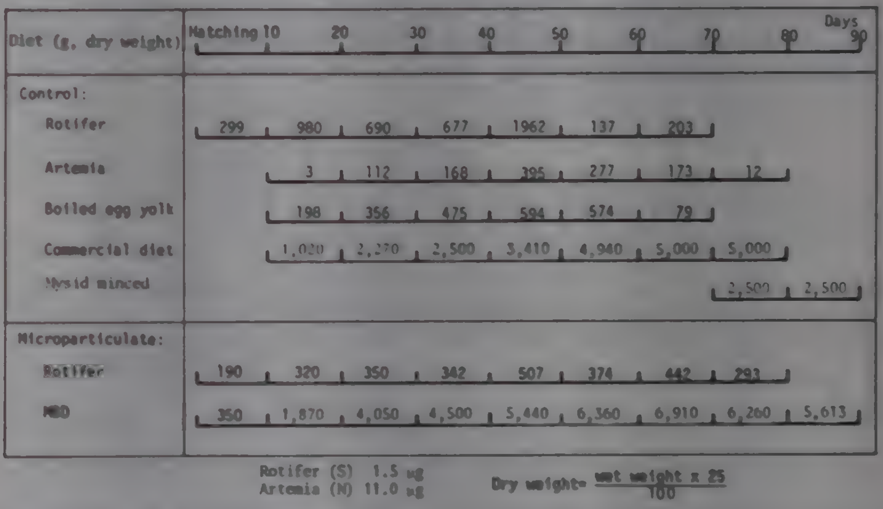
Diet (g, dry weight)
Hatching 10 20 30 40 50 60 70 80 90
Days
Control:
Rotifer 299 980 690 677 1962 137 203
Artemia 3 112 168 395 277 173 12
Boiled egg yolk 198 356 475 594 574 79
Commercial diet 1,020 2,270 2,500 3,410 4,940 5,000 5,000
Mysid minced 2,500 2,500
Microparticulate:
Rotifer 190 320 350 342 507 374 442 293
MBD 350 1,870 4,050 4,500 5,440 6,360 6,910 6,260 5,613
Rotifer (S) 1.5 μg
Artemia (N) 11.0 μg
Dry weight= wet weight x 25 / 100

Fig. 2. Diets and feeding amount used in each experimental group of 200,000 larval ayu.

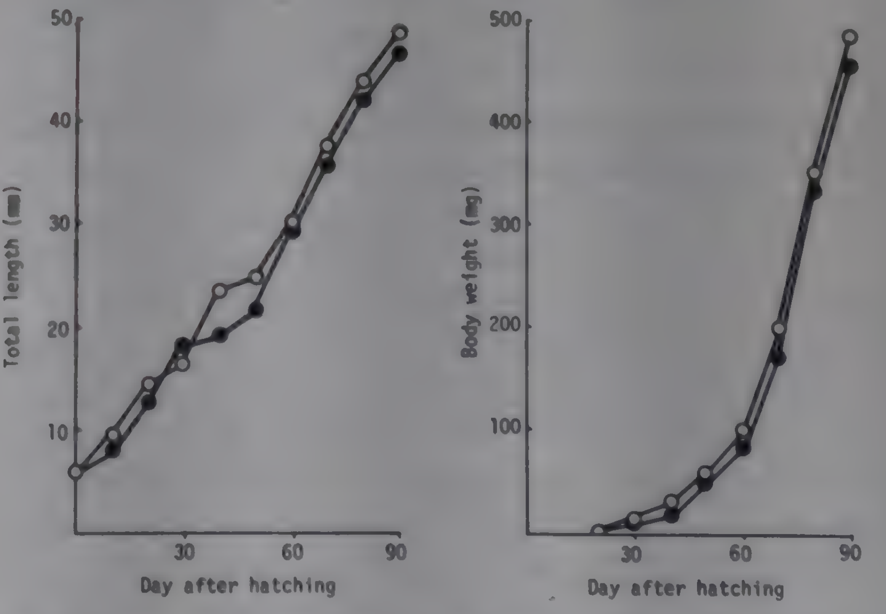

Fig. 3. Growth of larval ayu fed on microparticulate diets. ○ Control, ● Microbound diet (MBD).

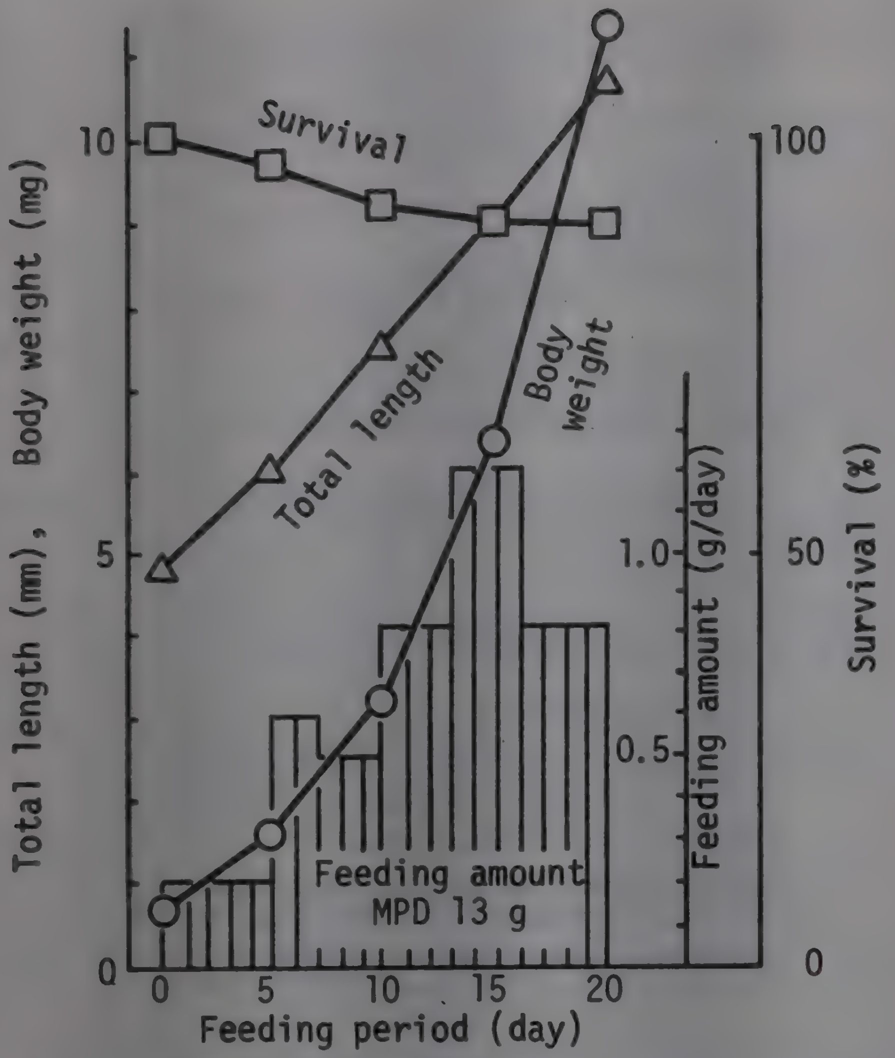

Fig. 4. Effect of microparticulate diet on growth, survival and feeding amount of carp.

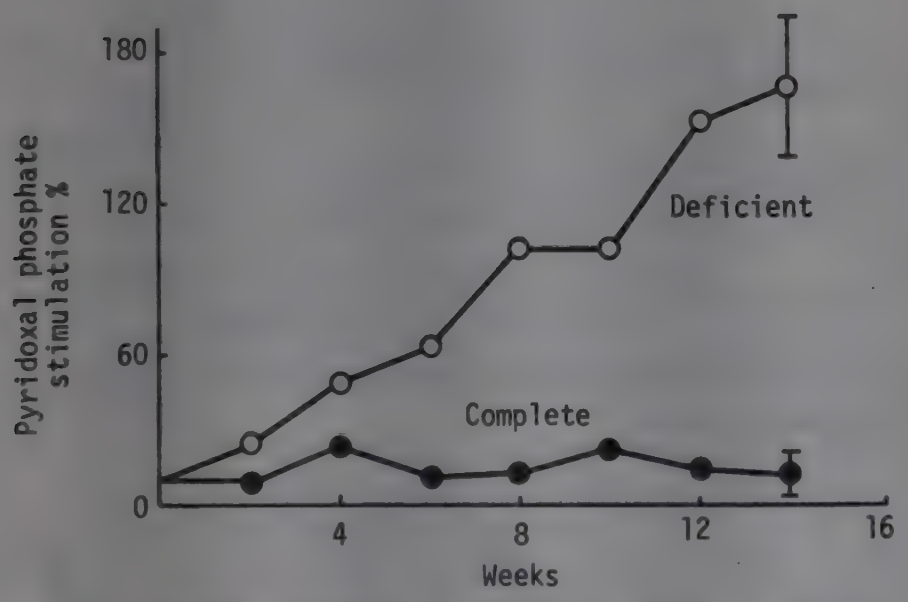

Fig. 5. Pyridoxal phosphate stimulation in plasma of rainbow trout fed complete or deficient diets.

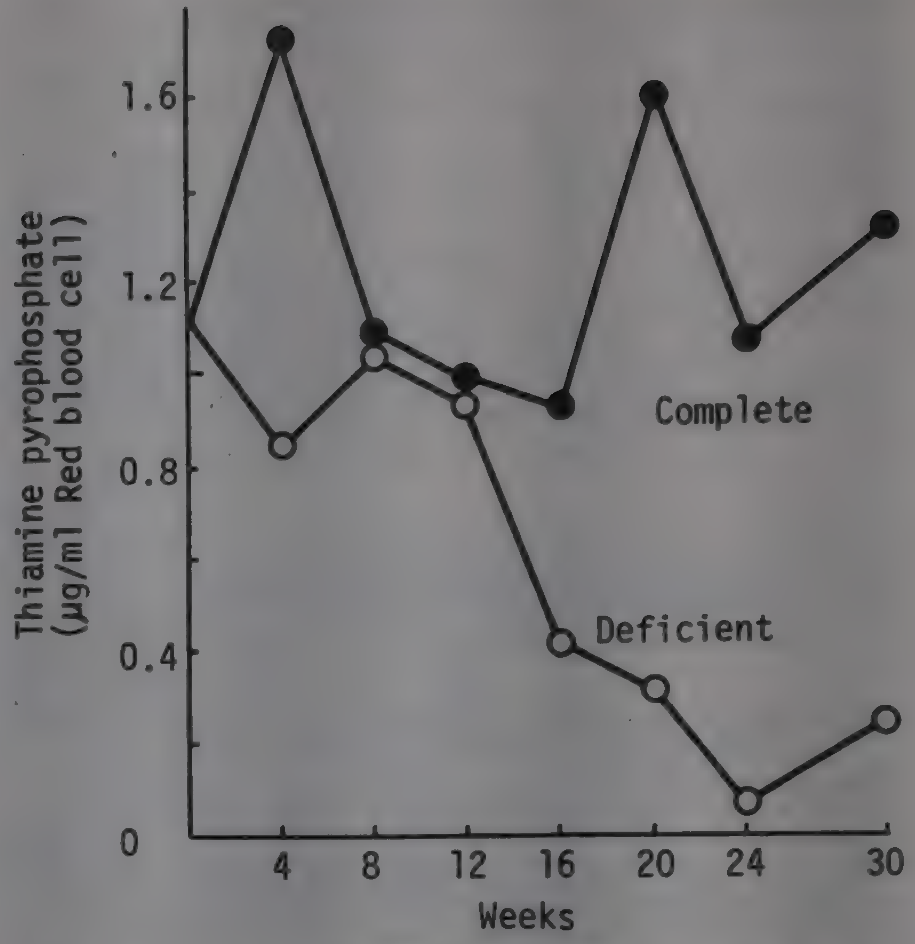

Fig. 6. Thiamine pyrophosphate content of red blood cell of rainbow trout fed complete or deficient diets.

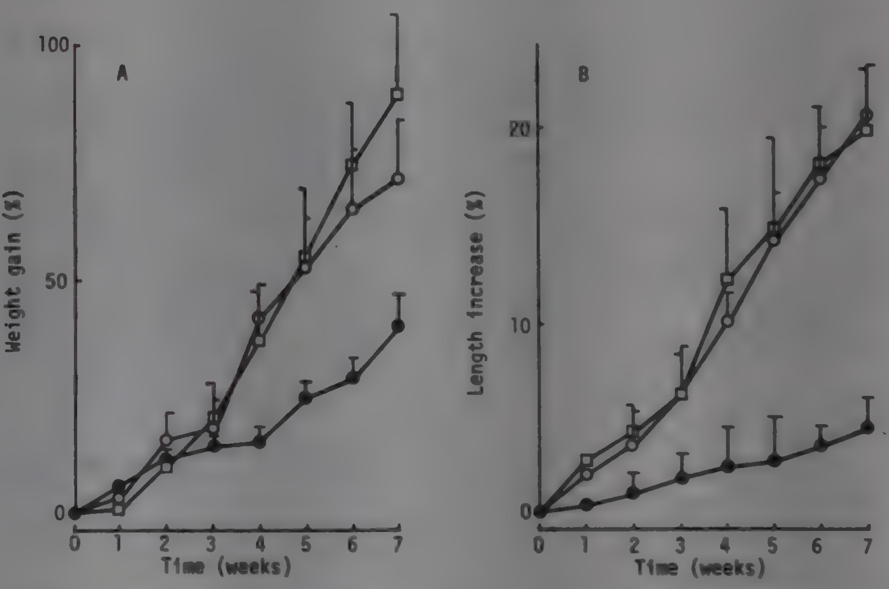

Fig. 7. sGH produced in *E. coli* (□, n = 8 fish per group) and natural sGH (○, n = 10). Both stimulate growth in weight (A) and length (B) of rainbow trout less than 1 year old. ○, control (saline-injected) fish (n = 10). Vertical lines represent ± SEM.

Concepts that Work: Some Advances in Tropical Fisheries Research*

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Abstract

Fisheries research is an applied discipline based in part on population and marine biology, with selected inputs from the social sciences. Fisheries research is successful when it provides concepts capable of helping fisheries management to make rational, explicit and enforceable resource allocation decisions. Successful concepts and models need not be as complex as the reality from which they are derived, and to which they are applied. Rather, successful concepts and models must capture and help express essential, and preferably quantifiable features of the reality that is modelled.

This contribution reviews a number of concepts, models and methods - some of them derived from approaches developed for high-latitude, single-species fisheries - which have proven useful in the context of the multispecies, multigear fisheries of tropical Asia. It is suggested that, overall, fisheries biologists working on tropical stocks do have the tools they need to do their job; the point now is to use them.

Introduction

Fisheries science investigates processes taking place within fish populations and within "populations" of fishing gears, but concentrates especially on the interface and interactions between fish and gears. Fisheries research is closely related historically and in various methods, models and assumptions, to marine biology and/or biological oceanography, but unlike them fisheries research is highly applied. While the success of fisheries research in a given country, for obvious reasons, cannot be assessed via the status of that country's fisheries, it remains true that success in our field can and must be measured by its relevance (potential or actual) to real fisheries.

It is my contention, which I will attempt to support in this contribution, that our discipline has matured to the extent that fishery scientists, including those working in tropical Asia, now have at their disposal an array of concepts and methods that "work", i.e., which can be used to answer most of the key questions explicitly asked by fishery managers and administrators, or implied by their general request for advice.

Russel's axiom as a research program

In 1931, when Russel published his landmark paper on overfishing, he effectively defined the field of fisheries biology as one concerned primarily with defining unit stocks and fisheries and their sizes, studying fish growth in and recruitment to these stocks, as well as investigating the natural mortality in and catches from investigated stocks (Fig. 1).

This research thrust, by putting emphasis on factors which, although often difficult to estimate precisely, at least can be approximated with sufficient accuracy, provided the conceptual basis upon which other authors, such as Beverton and Holt (1957) and Schaefer (1954, 1957) could build their models.

With regard to these models, two schools of thought can presently be defined. One advocates departure from the "classical population dynamics" approach it has spawned (Sharp and Csirke 1983; Bradbury and Reichelt 1981), suggesting instead that environmental factors or emergent system properties (i.e., holistic approaches) respectively, should receive greater emphasis. The other school, to which this author belongs, believes in refining, expanding and adapting the classical models. The basic reasons for the conservative stance are that environmentally driven ecosystem models of which exploited fish populations represent only a component often lack generality (i.e., applicability outside of the area for which they were developed) and that "holism" is probably more a general research mode than a concrete program of investigation around which, for example, student theses or postdoctoral studies can be structured.

Thus, when confronted with the need to translate Russel's axiom (in Fig. 1) to the multispecies situation prevailing, say, in Southeast Asian demersal fisheries, two approaches can be taken. The holistic approach would require here, I presume, treating each single species stock individually (but how does one get the data?) defining all interactions between species (how?), then developing a model that predicts the emergent properties of the system (presently an impossible task).

The other, "tinkering approach" would involve treating the whole species assemblage as if it were a single species (Gulland 1971), then fitting some production model to it (FAO 1978), or separating out subcommunities (Pauly 1979; Ralston and Polovina 1982; McManus, this vol.), and then applying standard models to them.

The second approach, as experience shows, besides providing neat theoretical insights, has the crucial advantage that the job gets done, and that management measures based on the results can be readily formulated. I believe that gross errors are less likely to occur in this "reduced" mode than if a large-scale (simulation) model is built from first principles (see also Larkin and Gazey 1982).

Growth studies: no real problems, just plain work

Growth is an important aspect of the dynamics of fish stocks. It is the only process which replenishes the biomass taken from a stock by fisheries or by predators, diseases and parasites. Growth rate estimates also provide the "chronological backbone" for mortality estimates. Quantifying growth, e.g., in the form of parameters of the von Bertalanffy growth equation is not anymore a scientifically daunting task. The main job for fishery scientists, especially in tropical Asia, is to acquaint themselves with current methods for assessing the growth rate of fish and exploited invertebrates, rather than to repeat the mistakes of often ill-informed experts from colder climates, many of whom still assert that tropical fish cannot be aged because they lack the scale or otolith annuli of herring and cod fame.

Among the methods ideally suited for the study of fast-growing small tropical fisheries (or various molluscs, including squids) are: (i) the analysis of daily otolith rings, discovered by Panella (1971) and confirmed by numerous scientists for all fish and squids so far examined, but still not used much in Asia (note the absence of papers on this topic among the 230 papers presented at this Forum); and (ii) detailed analysis of length-frequency data using computer-based methods, which are superior to the "Petersen method" documented about 90 (!) years ago (Petersen 1892), but which is still discussed by some colleagues in a fashion suggesting that it has never been improved upon.

Fig. 2 shows the application of one of these newer methods, the ELEFAN I program, to length-frequency data. As might be seen, the occurrence of a protracted spawning/recruitment season with two peaks per year has not prevented the program from identifying a single optimum growth curve for this data set. Note also that this method can correct for bias due to gear selection using information embedded in the original length-frequency data themselves, and also pick up seasonal growth oscillations which, incidentally do occur in tropical waters (Pauly 1982).

Growth studies using daily rings are very tedious and error-prone. Similarly, application of ELEFAN I to bad data can produce misleading results. This, however, is true for any method. The point here is that powerful methods (which can also be used concurrently) are available to estimate the growth of virtually any tropical fish. One should learn about and refine them by applying them.

Estimating mortalities: how good is good enough?

Fisheries biologists elaborating on Russel's axiom have, with regard to mortalities, the job of either estimating natural (M) and fishing mortalities (F) or, as is more usually done, estimating total mortality (Z) then subtracting either F (the older approach) or M (the newer approach) from Z to estimate the remaining parameter. One way fisheries biologists working in tropical Asia can go about this job is to estimate Z based on the size distribution of fish in representative catch samples, using either the mean length above a certain critical size (L') (Beverton and Holt 1956) or a length-converted catch curve (Pauly 1984; see Fig. 2). Then they can subtract from the estimate of Z the value of M obtained from an empirical formula (Beverton and Holt 1959; Pauly 1980) to obtain F.

Some are hesitant to use this approach, and I suspect that it might have to do with the feeling that the whole thing may be too simple to be correct. Let us not forget however, that in the North Atlantic, numerous stocks, e.g., of gadoids and herring, have been managed (or mismanaged, as the case might be) for decades based on values of "M = 0.2" and "M = 0.1", respectively, these values being passed from one stock to the other, from one paper to the other like a sacred incantation, and with no paper of which I am aware explicitly criticizing the practice.

It thus seems odd that Gulland (1984) should suddenly request scientists working in developing countries to use only values of M that have confidence intervals around them, and suggest that people should be particularly wary of M values obtained via the empirical equation in Pauly (1980).

Be as it may, fishery scientists, given appropriate samples can now straightforwardly split Z into its two components and then assess the relative impact of fishing and natural losses as causes of death in a given stock. Thus, we have here another concept that works.

Recruitment studies: dealing with concepts that do not (yet) work

Compared with growth and mortalities studies, recruitment studies on commmercial fish stocks in tropical Asia are extremely difficult and in a sense, one could argue that the recruitment-related concepts used in fisheries biology actually do not work, either in tropical Asia or in other parts of the world (see contributions in Sharp and Csirke 1983). The realization of this state of affairs has led, however, to a set of focused research activities, notably the International Recruitment Program (IREP) coordinated by UNESCO and FAO in the frame of their Ocean and Living Resources (OSLR) Program (Bakun et al. 1982). OSLR contributions on recruitment that are of relevance to the multispecies demersal and pelagic fisheries of tropical Asia are included in Yañez-Arancibia and Pauly (in press).

Although we do have basic problems predicting recruitment fluctuations, there are some concepts that work where our goal is to describe data sets. One of these simpler concepts is that of a "recruitment pattern", used to describe the seasonal oscillation of recruitment of fish into a stock, and which can be derived quite straightforwardly from length-frequency data (Fig. 2).

Catch, effort and cost data: the fishery biologist's workhorse concepts

Studying the growth, mortality and recruitment of the fish in a given stock allows one to use the yield-per-recruit model of Beverton and Holt (1957) or one of its derivatives to investigate the status of fishery. Pauly and Soriano (this vol.) provide examples of how such models can still be improved upon and made more relevant to specific situations, e.g., that of a demersal fishery exploiting small fish or shrimp. Yet, despite the adaptability of yield-per-recruit and other analytical models, it is on the surplus production model of Schaefer (1954, 1957) and its various derivatives (e.g., FAO 1978; Csirke and Caddy 1983) that most management advice in tropical Asia and other low latitude areas of the world will continue to be based.

This is simply due to the fact that surplus production models capture the essential aspects of any fishery, i.e.:

i) no catch (= surplus production) if you do not fish;

ii) a relatively large sustainable catch if you fish just right;

iii) a (much) reduced catch if you fish too much (Fig. 3).

A flood of literature exists which discusses the fine points of (ii) and (iii) [(i) is difficult to argue with], but this literature should not prevent us from realizing that we are, with items (i) to (iii) on extremely safe ground, and that any scientific advice based on these three points is bound to be better than the dangerously naive yet widespread assumption that more boats and more subsidies always mean that more fish will be caught.

The inexorability of this becomes even more apparent when one includes fishing costs into these considerations. In such cases, even skeptics can be convinced that subsidizing overfishing is, at least in the long run, a policy likely to lead to serious social problems (Smith 1981).

And yet it is here, with their best-founded, most self-evident concept that fisheries biologists and economists have least success. Indeed, translations of insights such as expressed in Fig. 3 (for which numerous well-documented examples exist in Asia) into a coherent policy aimed at reducing fishing effort are extremely rare, if not totally absent.

Possibly, estimating the size of actual or potential losses due to mismanagement for the fisheries of one country could help fisheries biologists, working in tandem with fisheries economists, to get their message across.

Unless these, and related "public relations activities" happen, it is difficult to conceive how, in the face of growing fish demand and growing need for export production, the fish resources of the world, and especially those of tropical Asia, will continue to feed us.

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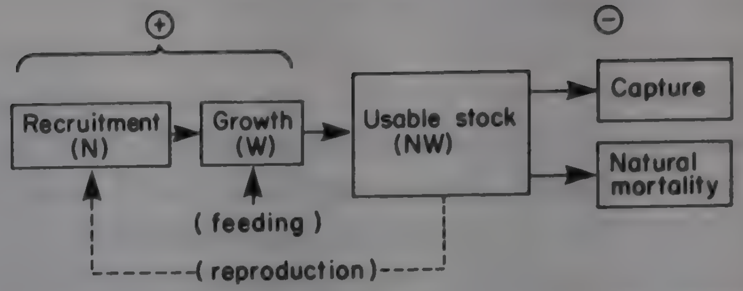

Fig. 1. Main factors investigated in stock-assessment:recruitment, growth (positive factors), capture and natural mortality (negative factors) and stock size. (N refers to numbers, W to weight). Feeding and reproduction, although also contributing to stock dynamics, are generally not considered in single-species stock assessment (adapted from Russel 1931).

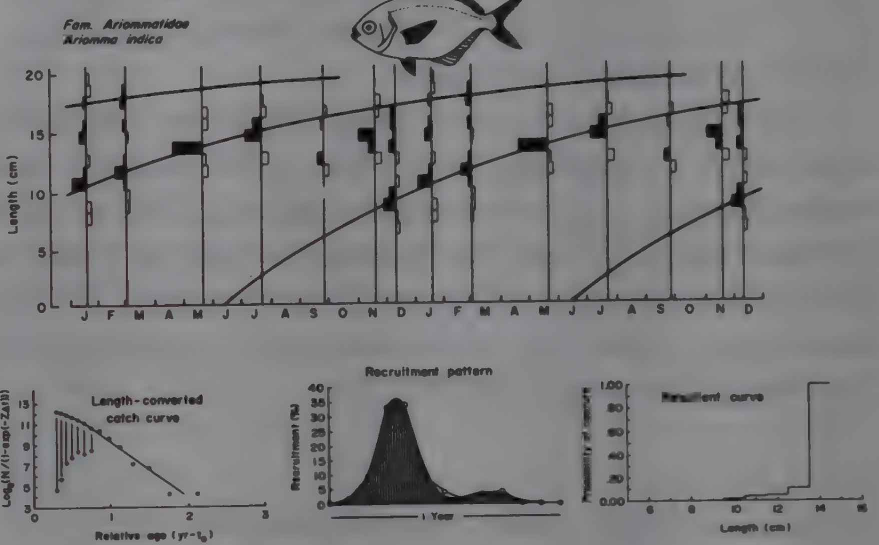

Fig. 2. Application example of the ELEFAN I and II programs to length-frequency data, sampled in 1981, on Indian driftfish from Ragay Gulf, Philippines. The following parameter estimates were obtained: L_{∞} = 21 cm, K = 1.1 (per year), M = 2.1, F = 3.3, mean length at first capture ≈ 13 cm (from Corpuz et al. 1985, with small modifications).

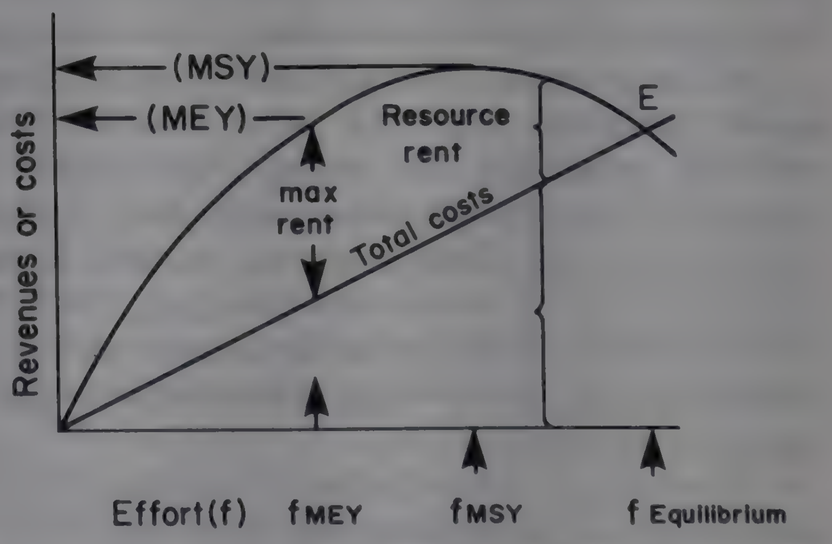

Fig. 3. An open-access fishery will tend to equilibrium (E) where total revenues become equal to fixed, variable and opportunity costs and no resource rent is earned. Note that beyond the level of fishing effort corresponding to MSY (f_{msy}), subsidies will have the effect of *decreasing* catches and total revenues from a fishery; note also that MEY (i.e., maximum rent) is reached at a level of effort smaller than needed for MSY (adapted from Smith 1981).

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*ICLARM Contribution No. 345.

Evaluation of the Growth Performance of *Oreochromis niloticus* Progenies in Freshwater Ponds

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Abstract

Evaluation of the growth performance of selected *Oreochromis niloticus* progenies was conducted in six 0.05-ha ponds at the Freshwater Aquaculture Center, Central Luzon State University, Muñoz, Nueva Ecija, Philippines. Two growth lines from the selection for growth at the center, high line (HL) and low line (LL) were stocked at 200 fingerlings per pond with an initial average weight of 0.33 g and 0.29 g, respectively. Growth performance, survival and sex ratio were monitored during the 90-day culture period.Experimental fish were subjected to a management scheme with just organic and inorganic fertilization using chicken manure and ammonium phosphate at the rate of 1,000 kg/ha and 50 kg/ha, respectively.At the end of the 90-day culture period, the high line group gave a higher mean weight of 119.33 g than the low line group with 100.37 g. Survival and sex ratio were comparable in both growth lines.

Introduction

Oreochromis niloticus is the most popular tilapia species cultured in the Philippines. Its fast growth, tolerance to poor environmental conditions and favorable breeding characteristics are only some of the traits which make *O. niloticus* a favorite fish for culture (Guerrero 1981) and an excellent candidate for a truly domesticated fish or "an aquatic chicken" (Maclean 1984). However, domestication of this fish is proceeding slowly with little effort to control genetic changes in economic traits or even the recognition that management procedures will lead to changes in the gene pool of the domesticated stocks. The unconscious husbandry practice of fish farmers have resulted in a deteriorating quality of tilapia fingerlings for culture and this is one of the factors that has hampered the expansion of the tilapia industry. One possible approach to this problem is to develop genetically-improved tilapia to optimize production.

A growing interest is now seen in fish genetics. This is triggered by both biological and economic reasons (Hedgecock et al. 1976). Efforts to improve the existing tilapia stocks in the country started with studies on the growth performance of various tilapia species/strains (Bautista unpublished data; Basiao unpublished data Guerrero et al. 1980; Kuo and Abella 1981).

This paper presents the results of an evaluation study on selected lines for growth of *O. niloticus* in earthen ponds.

Materials and Methods

Broodstocks from different sources were used as foundation stock for the selection program. The strains were Singapore from the Philippine Bureau of Fisheries and Aquatic Resources (BFAR) at Central Luzon State University (CLSU), Israel from BFAR at CLSU, Taiwan (imported by the International Center for Living Aquatic Resources Management in 1984 to BFAR at CLSU) and the Freshwater Aquaculture Center (FAC) strain. Females from each strain were crossed with a single male. Intrastrain crossing was done in plastic pools.

Offspring were collected from the breeding pools by seining at intervals of 10-14 days. Females were seined individually and their mouths inspected for eggs or fry which were removed for incubation or holding in the aquaria.

All spawns that had more than 100 fry surviving to free swimming stage, i.e., complete yolk-sac absorption, were stocked into 1-m^3 hapas installed in a single pond to minimize environmental differences. Each hapa represented one family with 100 fry. Sampling for weight and survival was done biweekly. In the first series of hapas, feeding was done by percent body weight of the fish in each hapa and adjusted after each sampling. The feeding rates were 100%, 50%, 30%, 20%, 15% and 10%. Feeding schedules in the second series of hapas were based on a growth curve of the five heaviest hapas in the first experiment. Every hapa was fed the same amount at a given age. These amounts were 100% (week 1), 70%

(week 2), 50% (week 3), 35% (week 4), 25% (week 5), 20% (week 6), 12% (week 8) and 10% (week 9 and after) of the growth curve estimated from the first experiment. Eight-week old fingerlings were transferred to 1-m^3 cages. Feeding fingerlings here was set at 10% of the body weight.

All families were subjected to selection for high and/or low growth lines at the 14-week sampling. Up to 20 males and 20 females were weighed for the selection. The two largest and/or smallest females were selected for the broodstock ponds. Fifty-eight families reached this selection stage.

The ponds for the growth phase study were prepared by following the FAC standard pond preparation. Ponds were fertilized with organic (chicken manure) and inorganic fertilizer (16-20-0) at the rate of 1,000 kg/ha and 50 kg/ha, respectively. Organic fertilizer was applied on a weekly basis while inorganic fertilizer was bi-weekly.

Two sets of stocking were made due to low production of fry of the selected broodstocks for the first few weeks. Ponds 5F and 5G were stocked on 13 September 1985 while ponds 5D, 5E, 4F and 4G were stocked on 28 October 1985.

Sampling was done monthly to monitor growth and survival. The same number of seine tows were made to collect samples.

Data were analyzed using completely randomized design.

Results and Discussion

After the 90-day culture period, the high line (HL) and low line (LL) groups gave an average final weight of 119.67 g and 101.36 g, respectively (Table 1). From this result it can be gleaned that the HL group was 18.31% heavier than the LL group. The daily gain in weight for HL was 1.33 g and 1.12 g for LL, although the HL showed a better performance than the LL. The difference in the average weight of the two lines was not significantly different (P 0.05) but the genetic improvement on this two-way selection cannot be discounted. A significant result on bidirectional selection for body weight of blue tilapia was reported by Bondari et al. (1983) while Moav and Wohlfarth (1976) produced an asymmetrical response on the selection for growth rate in common carp using the two-way selection.

Mean survival rate for HL and LL was 88.17% and 92.25%, respectively (Table 1). No significant difference was found in the survival rate between HL and LL.

Higher percentages of males were observed in HL (63.14) than LL (41.24); however, there was no significant difference in the percentages of males and females between the two lines.

Recommendations

Further evaluation of the selected lines, particularly the succeeding generation, should be conducted to find out the genetic gain from the selection process. Variations in ponds (e.g., primary production, physicochemical parameters) must be considered to minimize the effect of enviromental changes to the fish being evaluated. One way to do this is to grow the selected lines and the control in one pond, thus exposing the fish to the same conditions. This will require an efficient tagging method to mark the selected and control fishes.

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Table 1. Evaluation of selected growth lines of *Oreochromis niloticus* progenies.

					Sex ratio	
Treatment	Pond	Initial wt. (g)	Final wt. (g)	Survival (%)	Male (%)	Female (%)
High line	5F	0.31	120.83	81.50	69.10	30.89
	5D	0.37	107.46	94.00	59.46	40.54
	4G	0.29	130.73	89.00	60.87	39.13
Mean		0.32	119.67	88.17	63.14	36.85
Low line	5G	0.22	114.92	87.00	37.31	62.69
	4F*					
	5E	0.36	87.80	97.50	45.16	54.84
Mean		0.29	101.36	92.25	41.24	58.77

Table 2. Average body weight of selected line of *O. niloticus.*

			Culture period (weeks)			
Line	Pond	Stocking	2	4	6	8
High line	5F	0.31	15.15	73.83	101.04	120.83
	5D	0.37	17.78	57.01	80.44	107.46
	4G	0.29	16.20	76.12	89.73	130.73
Mean		0.32	16.38	68.99	90.40	119.67
Low line	5E	0.36	19.27	62.13	75.40	87.80
	5G	0.22	16.67	63.59	92.87	114.90
	4F*					
Mean		0.29	17.97	62.86	84.14	101.35

*Data from pond 4F were not used due to mixing of fish populations.

The Effects of Water Depth and Circulation on the Water Quality and Production of *Penaeus monodon* in Earthen Ponds*

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Abstract

Successful high intensive shrimp grow-out schemes typically use deep ponds (1-2 m) together with aeration/circulation. Little is known, however, why deep ponds are more productive than shallow ponds. It is important to understand the water quality and production dynamics of ponds of different depths to develop appropriate shrimp culture methods. The effects of water depth and circulation on the production of the giant tiger shrimp, *Penaeus monodon*, in 0.1-ha earthen ponds were tested in a 3 x 2 factorial experiment, with three depth treatments (0.5, 1.0 and 1.5 m) and two circulation regimes (daytime circulation and uncirculated). Stocking density was 4 postlarvae/m^2. Production and survival were determined after five- and four-month culture periods during the dry and wet seasons, respectively, in 1985. Water circulation positively influenced primary productivity, decreased the surface temperature, and reduced stratification of temperature and dissolved oxygen. Water depth significantly affected almost all water quality parameters, the deeper ponds producing shrimp of significantly larger size. However, there were no treatment effects on shrimp production due to an inverse relation of survival and average size. It can be said that water depth and circulation profoundly affect the water quality of brackishwater shrimp ponds, but that the effects on shrimp production are not apparent at the stocking density used in this experiment. Further tests at higher stocking densities are necessary to establish the causal relationships of water depth, survival and average size of shrimp.

Introduction

One factor that contributes largely to the success of semi-intensive and intensive penaeid grow-out schemes is effective water management. There are several water management techniques for penaeid pond culture, all involving varying water depths and circulation/aeration methods (Kungvankij 1985). Average pond water depth and water movement are two important factors that can affect numerous aspects of pond environments. These include effects on the heat budget, thermal and chemical stratification, minimum oxygen concentrations and ultimately the growth and survival of the shrimp.

The Taiwanese are well known for their outstanding achievements in the development of penaeid pond grow-out systems (Liao 1985). These schemes depend heavily on deep water and substantial circulation/aeration and yet there appears to be a lack of basic information regarding the environmental dynamics of these systems. It is important to understand these pond dynamics in designing appropriate grow-out strategies for shrimp in countries with economic situations different from those of Taiwan. For example, energy intensive systems are not as prohibitive in Taiwan because of low energy costs, whereas energy costs in the Philippines often make intensive culture systems uneconomical. Furthermore, Taiwanese shrimp ponds are usually deep, as much as 1.8 m, (Fast et al., unpublished data) while the majority of brackishwater fishponds in many Southeast Asian countries are as shallow as 0.5 m. It maybe improbable, therefore, for the shallow ponds in the Philippines to be used for intensive shrimp grow-out using Taiwanese techniques. The purpose of this study was to evaluate the effects of different water depths and water circulation regimes on water quality as well as growth and survival of the giant tiger shrimp, *Penaeus monodon*, in earthen ponds.

Materials and Methods

A 3 x 2 factorial design was used to test the effects of depth and circulation on water quality and shrimp production in earthen ponds. Eighteen 0.1-ha earthen ponds were used, with depth treatments of 1.5, 1.0 and 0.5 m. Three ponds in each depth treatment were subjected to artifical circulation during the daytime (6 a.m to 5 p.m.), while the other three were not. Circulation was done by a device consisting of a 1/8-hp electric motor, driving a submerged 46-cm fan-type impellor at 86 rpm similar to those described by Fast et al. (1983).

The experiment was conducted twice, with a 150-day grow-out during the dry season (December 1984-April 1985) and a 120-day grow-out during the wet season (August-December 1985). The ponds were prepared by draining, drying, applying agricultural lime and chicken manure at 2 t/ha to each, and then gradually flooding to grow benthic algae *(lablab)*. In each trial, *P. monodon* postlarvae were stocked at 4/m^2. The average weight of initially-stocked postlarvae during the dry season was 4.7 mg and during the wet season 2.1 mg. Feeding rate was at 10% average body weight during the second month of grow-out, 8% during the third month and 4% during the final month. Formulated feeds were not applied during the first month. Water exchange was twice a month, depending on the occurrence of spring tides, at a rate of 50% of total water volume each time. Additional water exchange through pumping was sometimes necessary during the later stages of grow-out because of low dissolved oxygen levels. Emergency aeration with a 1/2-hp paddle wheel aerator was also used occasionally.

Salinity was measured daily with a refractometer. Dissolved oxygen and temperature at varying depths at different times of the day were measured three times a week with a YSI oxygen meter. Visibility with a secchi disk and pH with a Corning pH meter were also measured three times a week. Chlorophyll *a* concentrations, un-ionized ammonia, nitrates, nitrites, dissolved orthophosphate and plankton levels were monitored twice a month. Chlorophyll *a* concentrations were determined according to Lind (1974). Concentrations of un-ionized ammonia, nitrates, nitrites and dissolved orthophosphate were determined with the methods of Strickland and Parsons (1976). Un-ionized ammonia and dissolved orthophosphate were analyzed by the phenol hypochloride and ascorbic acid methods, respectively. Phytoplankton were counted with a hemocytometer, zooplankton with a Sedgewick rafter. Prawn growth was monitored once a month by weighing a subsample of shrimp from each pond.

The SYSTAT package (Wilkinson 1984) was used for all statistical analysis. Water quality data were averaged monthly corresponding to intervals between the shrimp sampling dates. A two-way ANOVA factorial analysis with replication per sampling was done on the water quality parameters. A stepwise multiple regression analysis was done to determine which of the variables best predicted the average size of shrimp per sampling (i.e., the growth of shrimp). All the water quality variables listed in Table 1 were initially entered in the model (both alpha to enter and alpha to remove levels were $p = .15$).

The analyses of growth and harvest data for the dry season are not presented here because the large number of finfish intruders in the ponds made it difficult to assess treatment effects on *P. monodon* alone. During the wet season, ponds were treated with an ichthyocide (teaseed cake) controlling intruders to negligible levels (Minsalan and Chiu, this vol.). A two-way factorial ANOVA was done on the wet season harvest data.

Results and Discussion

Most water quality values changed significantly during the grow-out period, with the exception of un-ionized ammonia concentrations (Table 1), which were also unaffected by any of the depth or circulation treatments. It is possible that ammonia nitrogen is readily metabolized in brackishwater ponds and rarely attains high concentrations. Seasonal weather changes are most probably the reason for changes in pond temperature and salinity during the grow-out period. Changes in productivity and nutrient concentrations with time were perhaps due to an increased feed loading during the grow-out. These changes were exemplified by increases in secchi disk depth, dissolved oxygen, chlorophyl concentration, phytoplankton, zooplankton, nitrates, nitrites and reactive phosphorus.

Most of the water quality parameters were significantly affected by the depth treatments (Table 1). Those variables related to productivity (secchi disk depth, morning and afternoon oxygen concentrations, Chlorophyll *a*, phytoplankton and zooplankton) and nutrient concentrations (nitrites, nitrates and reactive phosphorous) were significantly higher in the shallower pond. This is undoubtedly due to lesser volumes of water in the shallow ponds, since all ponds received equal feed applications. Cole and Boyd (1986) have shown that average concentrations of such water quality parameters as Cholorphyll *a*, nitrite nitrogen and chemical oxygen demand increase with increasing feeding rates. A similar relationship is expected with equal feed applications to ponds with decreasing water volumes. The shallow ponds also proved to be less stable environments with regard to diurnal temperature fluctuations. These ponds had significantly higher afternoon and significantly lower morning temperatures (Table 1).

There were few significant treatment effects due to circulation. Daytime circulation did, however, appear to lower the surface temperature of the pond. There was significantly lower afternoon temperatures during the dry season and significantly lower morning temperatures during the wet season in circulated ponds. Daytime circulation also appeared to increase primary productivity as Chlorophyll *a* concentrations were higher in circulated ponds during the wet season. This increase in primary productivity due to circulation is also evidenced by higher dissolved oxygen concentrations in the afternoons. Daytime circulation also substantially decreased thermal and oxygen stratification in the pond throughout the diurnal cycle.

In both trials, the average shrimp size per sampling was significantly higher in the deeper ponds (Table 1). The faster growth of shrimp in the 1.5-m and 1.0-m deep ponds than in the 0.5-m deep ponds during the wet season trial is illustrated in Fig. 1. During the wet season, shrimps were larger in circulated pond water and significantly larger in circulated water in deeper ponds (Table 1). Growth, therefore, appears affected by both water depth and circulation.

Depth was retained by the stepwise regression procedure as a significant indicator of shrimp growth during the wet season (Table 2). Salinity was also retained in the model in view of its importance to the growth of *P. monodon*. Various other water quality parameters were also retained in the model which reflect the productivity (morning and evening dissolved oxygen and phytoplankton densities) and feeding rate (ammonia and nitrates) influences on shrimp growth.

There were significant effects of water depth on both size and survival of *P. monodon* (Table 3). These dependent variables were, however, inversely related. Size was larger but survival was lower in deep ponds. A regression of size versus survival was significant ($y = 125.3-1.8x$, probability regression F ratio = .002). This inverse relationship explains the lack of significant treatment effects on total production (i.e., the deeper ponds had larger but fewer shrimp while the shallower ponds had smaller but more numerous shrimp). An analysis of covariance of size of shrimp versus the depth treatments with survival as covariate showed no significant ($p = 0.477$) depth effects on size of shrimp.

It is difficult to evaluate the effects of water depth on the growth of *P. monodon* in view of the inverse relation of size and survival during the wet season grow-out period. It is possible that survival is dependent on water depth and that shallow ponds would be expected to have higher survival, given approximately equal feeding rates in all ponds. If this is so, and if size is inversely and causally related to survival, then it may not be necessary to use deep ponds to obtain a particular yield level. It is also possible, however, that the survival patterns observed during the wet season trial are due to some factors unrelated directly to the depth treatments.

If water depth, average size and survival are causally related as the results from the wet season trial indicate, this scheme may be useful only at the stocking density used in this study ($4/m^2$). It is clear that depth and circulation affect many aspects of the pond environment. It may be that at stocking densities for more intensive penaeid culture, these water quality differences could substantially influence overall production. It would therefore be useful to test these treatment effects at high stocking densities.

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Table 1. Water quality parameters and growth of prawn.

	Dry season				Wet season			
Variable	Time	Depth	Circ	Depth xCirc	Time	Depth	Circ	Depth xCirc
Secchi depth	**	**			**	**	**	*
Temp. A.M.	**	**			**	**	— *	
Temp. P.M.	**	—**	—**	*	**	— *		
D.O. A.M.	**	**		*	**	**		*
D.O. P.M.	*	—**	**	**	*	—**	*	*
Salinity	**	—**			**			
Ammonia								
Nitrates	**	— *			**			
Nitrites	**	—**			**	—**		
Phosphorous	**				**	—**		
Chlorophyll a	**	—**			**	—**	*	
Phytoplankton	**	— *			*			
Zooplankton	**	—**			*			
Average size	**	**		*	**	*	*	*

**Significant at P < .001; *Significant at P < .05; Blank—not significant.
—Higher values in shallower ponds or higher values in ponds without circulation.

Table 2. Indicator variables for prawn growth during the wet season.

Dependent = log (average weight)		
Coefficient	Variable	Probability*
2.663	Constant	.000
0.610	Time	.000
0.012	Depth	.000
−0.229	D.O. A.M.	.000
0.052	D.O. P.M.	.015
−0.109	Salinity	.000
2.504	Ammonia	.005
0.398	Nitrates	.000
0.000	Phytoplankton	.111

*2 tail tests.

Table 3. Average weight, survival and total production per treatment, wet season trial.

Treatment Depth (m)	Circulation	Average weight (g)	% Survival	Total production (kg/ha)
1.5	Yes	33.2	65.9	873.3
1.5	No	33.9	57.3	777.6
1.0	Yes	35.7	58.8	829.8
1.0	No	28.2	77.0	875.9
0.5	Yes	28.4	78.3	887.9
0.5	No	26.3	80.5	861.1

Two-way factorial ANOVA:
Probabilities associated with the F-ratio

Treatments	Average weight	Survival	Total production
Depth	.018*	.009*	.775
Circulation	.076	.335	.858
Depth x Circ.	.125	.047*	.597

* = significant.

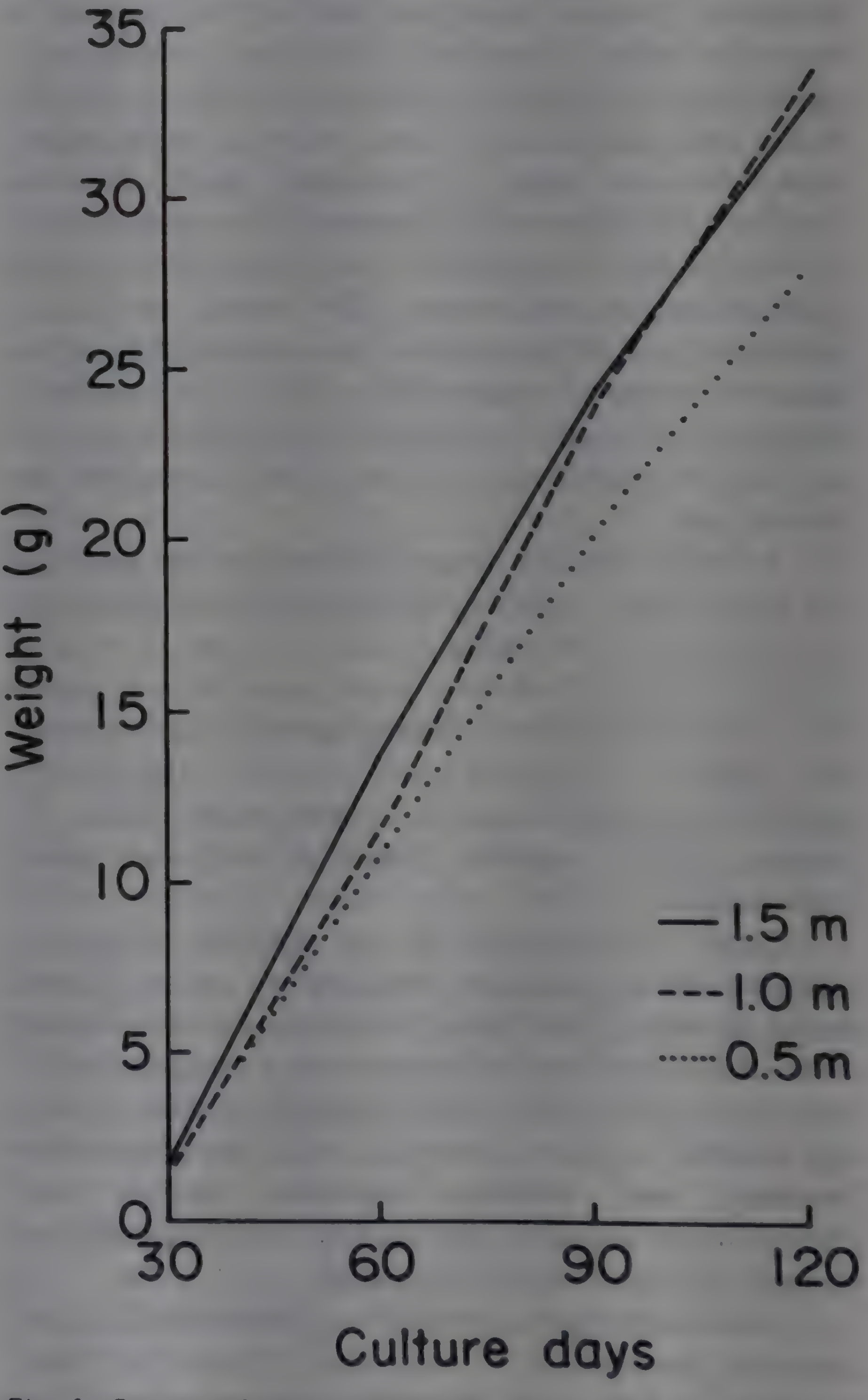

Fig. 1. Growth of prawn per depth treatment for wet season.

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The Prospects of the Grass Shrimp Culture Industry in Taiwan[1]

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Abstract

Grass shrimp *(Penaeus monodon)* culture is a rapidly growing, profitable industry in Taiwan. The total annual production of grass shrimp increased from less than 1,000 t in 1976 to more than 30,000 t in 1985. The economic importance of shrimp culture in Southeast Asian countries has also been widely recognized. Continued development and expansion of the industry might eventually lead to competition for markets.

The culture methods for extensive, semi-intensive and intensive culture systems in Taiwan are described and their economics analyzed. Speculations are made on strategies to ensure the continued success of the industry in Taiwan.

Introduction

The grass shrimp, *Penaeus monodon*, is universally recognized as one of the most important cultured species, particularly in tropical Southeast Asian countries. It is a fast growing, euryhaline, omnivorous and hardy species well known for its delicious taste and profitable marketability. The technique for the artificial propagation of the grass shrimp was first developed in Taiwan in 1968 (Liao et al. 1968). Formulated shrimp feed was successfully developed ten years later. These major technological breakthroughs have contributed to the rapid growth of the shrimp culture industry, which has played an important role in the economic development of Taiwan. The total annual production of grass shrimp has increased from 61 t in 1968 to 110 t in 1977 and 30,000 t in 1985. The number of shrimp hatcheries has also increased from one in 1968 to more than 1,500 in 1985.

In Taiwan, shrimp were cultured extensively with milkfish for many years. Before the first of several different shrimp species successfully underwent artificial propagation in 1968, seed stock was collected exclusively from the wild. The annual production of postlarvae increased from 1.2 million in 1968 to about 3 billion in 1985. In the past two decades, as the seed stock became widely available in sufficient quantities and the culture technology steadily developed, shrimp culture has progressed from polyculture to monoculture and from extensive to highly intensive culture. The scale of culture operations has expanded greatly, from small family-run farms to large-scale corporate-run businesses, although the former are still common.

This paper highlights the status of the shrimp culture industry in Taiwan and the major factors responsible for its production costs and suggests a strategy for its future development.

Shrimp Culture Systems

Three types of culture systems currently used reflect the evolution of shrimp culture over the last two decades. They are extensive polyculture (Type A), semi-intensive monoculture (Type B), and intensive monoculture (Type C). These culture systems are described in Table 1. Most farms are on the southwestern coast of Taiwan.

The primary culture commodity in Type A has been milkfish with grass shrimp being of secondary importance and stocked at very low densities, below 10 postlarvae (PL)/m^2.

The Type B system consists of culture ponds converted from shallow milkfish ponds with minor modifications such as the installation of a water-supply system and aerators. In most cases, stocking density ranges from 10 to 30 PL/m^2.

Most Type C systems were originally eel-culture ponds upgraded and equipped with better water supply and drainage and aeration through paddle wheels. Eel culture declined due to scarcity of elvers and the fall in eel prices.

The significant difference among the three types of culture systems is their varying degree of manageability. A more manageable system helps achieve a higher survival rate (Fig. 1). Given the same stocking density, the survival rate is determined primarily by environmental conditions. The Type C culture system was expected to produce higher yields because of the higher survival rate. From a 1984 survey the capacity of each system appeared limited; the survival rate declined sharply with excessive stocking densities, currently ranging from about 10 to 100 PL/m^2. Recent progress in culture technology has proven, however, that a survival rate higher than 80% can be attained as a matter of routine, provided the culture environment is properly managed to prevent disease and other unfavorable conditions.

Infrastructure

Hatchery operators can be classified into: (a) nauplii producer, whose main activities include broodstock husbandary, controlled maturation through eyestalk ablation and production of nauplii; (b) postlarvae producer primarily concerned with raising the nauplii through the postlarvae stage (PL11/13); and (c) nursery operator who raises postlarvae (PL 11/13-PL 20/22) to insure higher survival during the initial phase of stocking in grow-out environments.

In addition to *Skeletonema* and artemia nauplii, more than 20 types of artificial diets are presently used. The culture of larval food, particularly *Skeletonema*, is time consuming; thus the supply of *Skeletonema* and other algae has recently become a specialized business and a profession by itself.

All the necessary logistical support for shrimp culture is readily available in the local market. There are more than forty shrimp feed companies and this figure is expected to increase to almost 100 in the near future. In many cases, the products can be delivered directly to the shrimp farm.

Through the continued refinement of broodstock husbandry techniques, noteworthy progress has been made in the controlled maturation and efficient use of gravid female shrimp. To meet the increasing demand for broodstock, gravid females have been imported from various sources throughout Southeast Asia. Stock release projects have also been implemented for several years.

Production Economics

In the economic analysis of shrimp culture operations, there are several reasons why most shrimp farmers usually disregard the costs of land, labor and depreciation of their facilities for pond construction. First, farmers own their land and business and members of the farmers' family are the sole source of labor, with the exception of part-time workers, such as the harvesters. Second, because most shrimp culture operations were converted from eel culture farms used for more than five years, depreciation of pond construction is not included in cost accounting. In newly constructed culture systems, whether owned by an individual farmer or by a corporation, the abovementioned costs should be included.

In 1984, fifty shrimp farmers were interviewed and their production costs and profits for that year were analyzed. Nine factors were included in the analysis: (1) type of culture system; (2) years of culture experience; (3) culture area; (4) pond depth; (5) stocking density; (6) aerator coverage; (7) cost of formulated feed; (8) cost of supplemental food (i.e., trash fish); and (9) cost of chemicals.

The costs of formulated feed and the stocking density were the two most important factors affecting profits as determined by covariance and multiple regression analyses. The following equation fits the data collected.

$$\text{Profit} = -32.2003 + 3.5984 \times SD - 0.8835 \times FC$$

where SD represents stocking density PL/m^2 and FC represents the cost of formulated feed/m^2.

The production costs for a pond with a stocking density of 25 PL/m^2 is shown in Figs. 2a and 2b. Each production cost factor (feed, postlarvae, labor, pond maintenance, interest, depreciation, utilities, etc.) is closely correlated with the final survival rate. With the exception of formulated feed costs, all factors play a decreasingly important role as the final survival rate increases while formulated feed costs become of increasing importance. When land, labor and depreciation costs are not included, formulated feed accounts for 52.6% and 21.71% for survival rates of 80% and 20%, respectively.

The relationship between survival rate and stocking density is important in determining profitability and was analyzed for the three culture systems. Results (Fig. 1) show that survival rate is inversely related to the stocking densities.

Utility costs are mostly for electrical energy needed to power the pumps to change water and paddle-wheel aerators. Increases in energy consumption are related to stocking densities (Fig. 3). The use of treatment chemicals is also related to the shrimp stocking density.

The profitability of shrimp culture under three culture systems is further analyzed in Figs. 4a, 4b and 4c.

The breakeven points for the three types of culture systems, based on the relationship between the stocking density and survival rate are illustrated in Fig. 5. This provides the guidelines with which to manage profitably a culture business. It is obvious that higher survival rates are necessary to cover the costs of land and construction of the pond and facilities in a commercial operation.

Return on Investment

Information on production costs and profits enables investors to forecast their return on investment (ROI). Based on the 1984 culture performance, production costs have been estimated and related to the intensity of culture and survival rate. The estimated survival rate (SR), cost of feed (FC), cost of chemicals (CC) and cost of electricity (EC) were calculated from the following equations:

$$SR\ (\%) = 71.50 + 1.655 \times SD - 0.044\ (SD)^2$$

$$FC\ (\text{in NT\$/ha}) = 74.26 + 0.86 \times SD$$

$$CC\ (\text{in NT\$/ha}) = 6{,}600 + 8.00 \times SD$$

$$EC\ (\text{in NT\$/ha}) = 27{,}960 + 1{,}170 \times SD$$

The following assumptions were made to project the ROI at varying stocking densities.

Wage for a skilled technician	NT$ 20,000/year
Sale price of grown shrimp	NT$ 235/kg
Price of postlarvae	NT$ 600/1,000 PL
Depreciation lifetime	5 years
Land cost	NT$ 135/m2
Number of harvests/yr	2
Annual interest rate	10%

The trend of the ROI curves under varying degrees of investment for the construction of the ponds and facilities is shown in Fig. 6. The required stocking density at the maximum ROI varies with the construction cost. Higher investments in the construction of facilities result in better ROIs because of the higher survival rates attained due. This suggests that profits from shrimp culture are sensitive to technological inputs.

Strategies for Future Development

The most critical technological inputs in shrimp culture are: (1) nutritionally-balanced formulated feed for nursery and grow-out; (2) techniques and systems for efficient seed production; (3) methods for the proper use and application of chemicals/drugs for the prevention and treatment of diseases; and (4) management systems and methods for the effective maintenance of the culture environment.

As hatchery and culture technologies are upgraded, world shrimp production is expected to continually increase. If shrimp producers are to maintain their market share they must adopt more efficient culture methods. Therefore, they will have to monitor closely the costs of their most significant inputs, which are feed, postlarvae and utilities, in order of importance. Steps must also be taken to broaden the base of demand for shrimp.

It is essential that research efforts continue to maximize technological inputs while reducing their costs, especially on: (1) nutritional and dietary requirements; (2) continued refinement of existing feed formulae; (3) maximum utilization of locally-produced feed ingredients; (4) improving larval rearing techniques; and (5) developing better techniques for the prevention and treatment of diseases.

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Table 1. Prawn culture systems in Taiwan.

Description	Type A	Type B	Type C
Stocking density (postlarvae/m^2)	under 10	10-30	above 30
Type of culture	polyculture	monoculture	monoculture
Pond size (ha)	1-3	1/2-1	1/4-1/2
Depth (m)	0.3-0.4	0.6-1.5	above 1.0
Dyke	earthen	earthen	concrete or brick
Slope	1 : 1.5	1 : 1	vertical
Pond bottom	earthen/sand	earthen	earthen/sand or gravel
Water supply	tidal	tidal or pumping	pumping
Drainage system	not controlled	partially controlled	controlled
Paddle wheel (no./ha)	not installed	4 or more	8 or more

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[1] Contribution B No. 41 from the Tungkang Marine Laboratory.

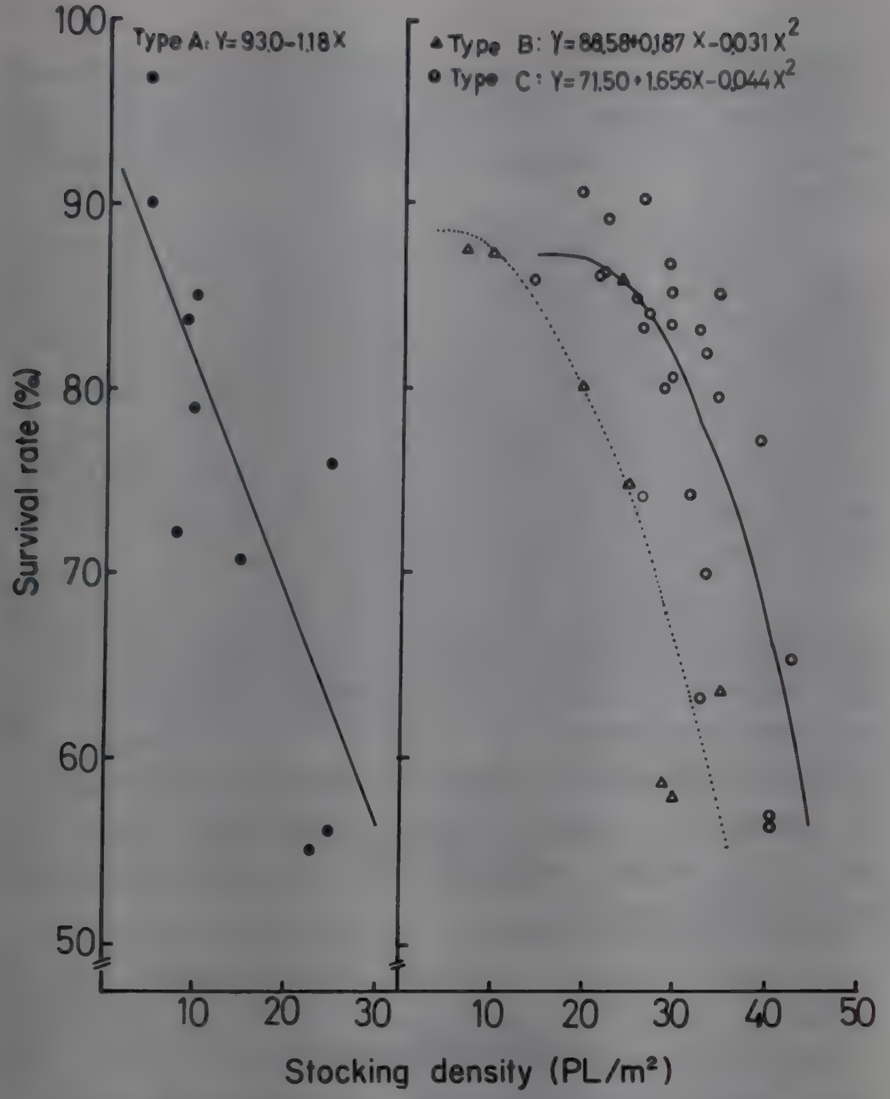

Fig. 1. Survival related to stocking densities under three different culture systems.

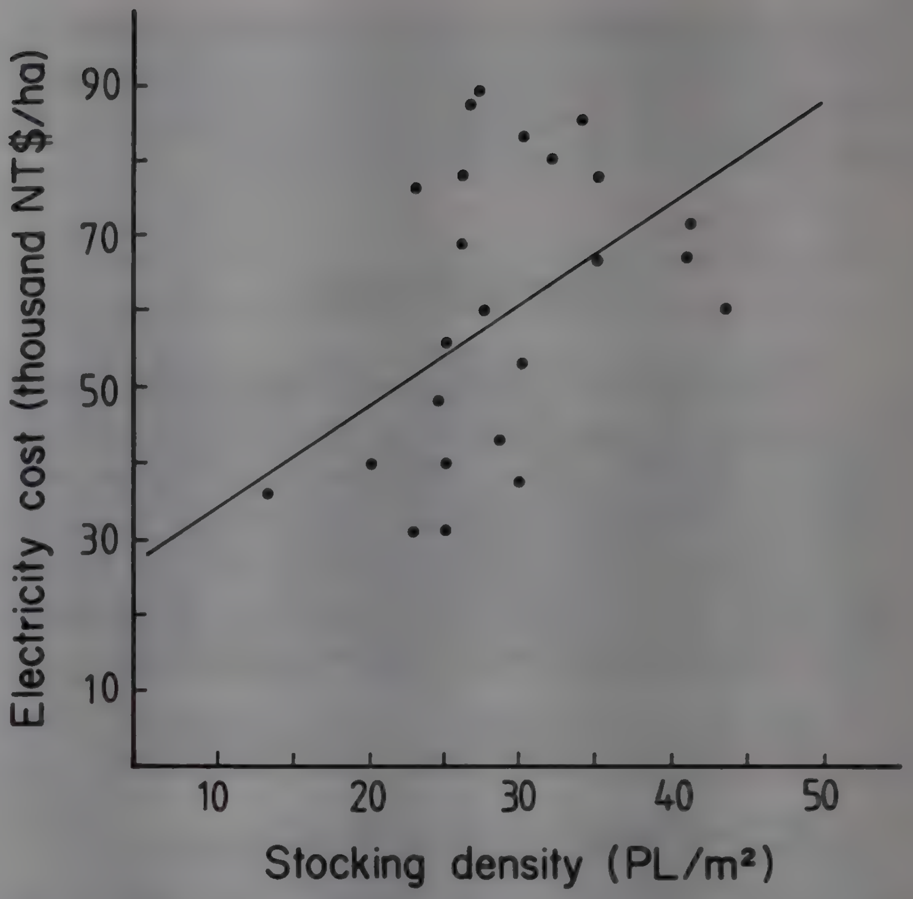

Fig. 3. Relationship between the electricity cost and stocking density.

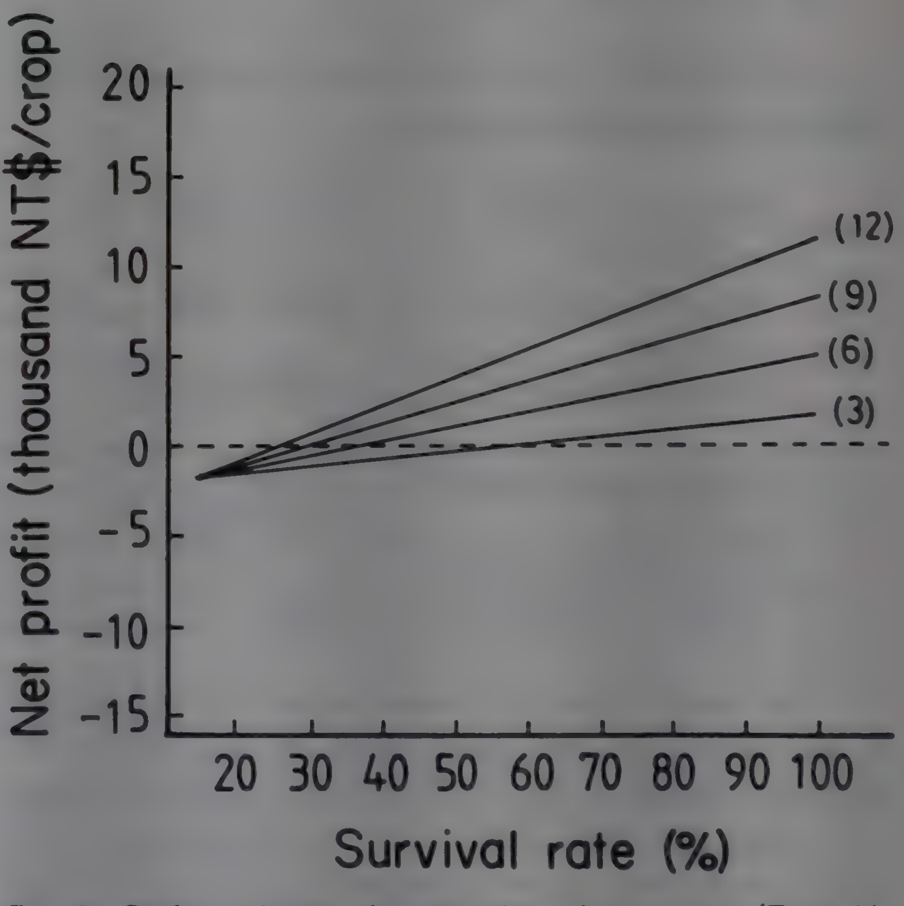

Fig. 4a. Profit projection for extensive culture system (Type A). The stocking densities are in parentheses.

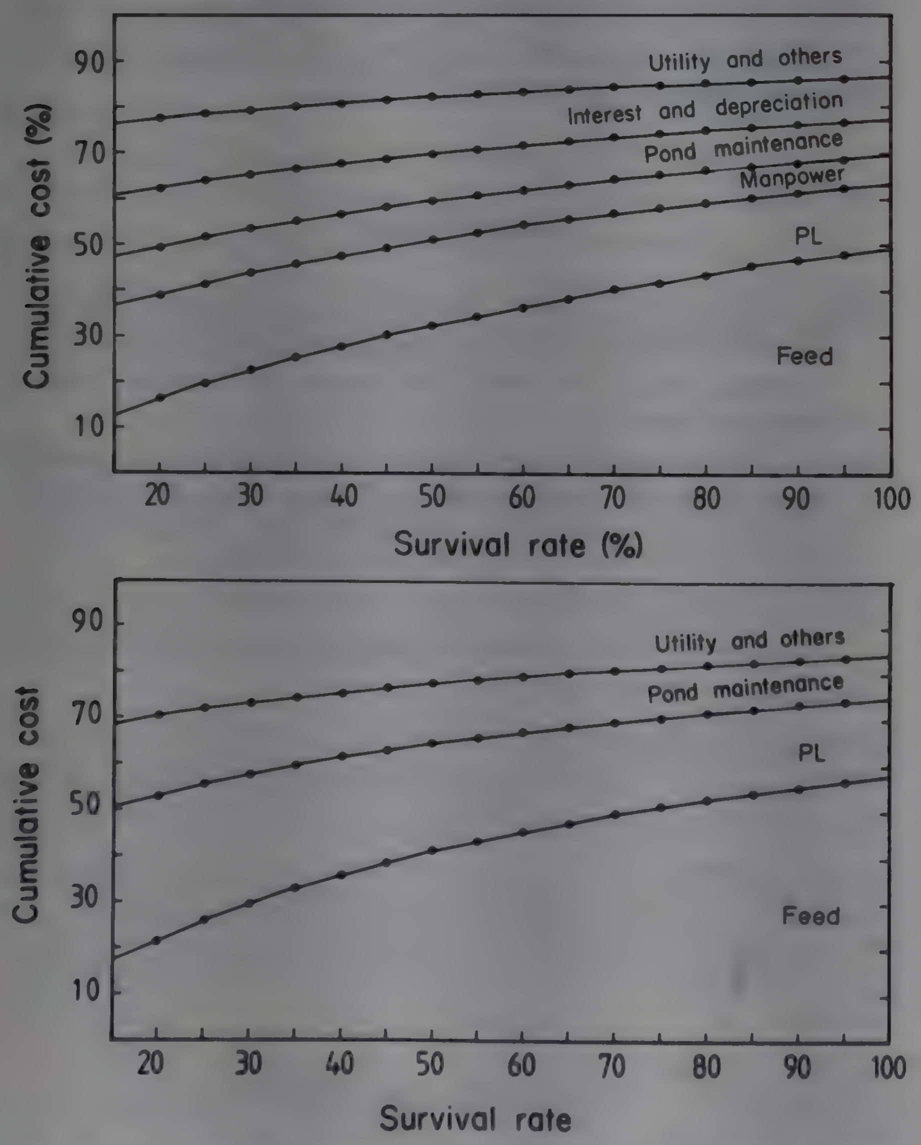

Fig. 2a and 2b. Production cost breakdown exemplified at the stocking density of 25 PL/m^2. The costs for land, pond and facility construction and manpower are not included in the production cost analysis.

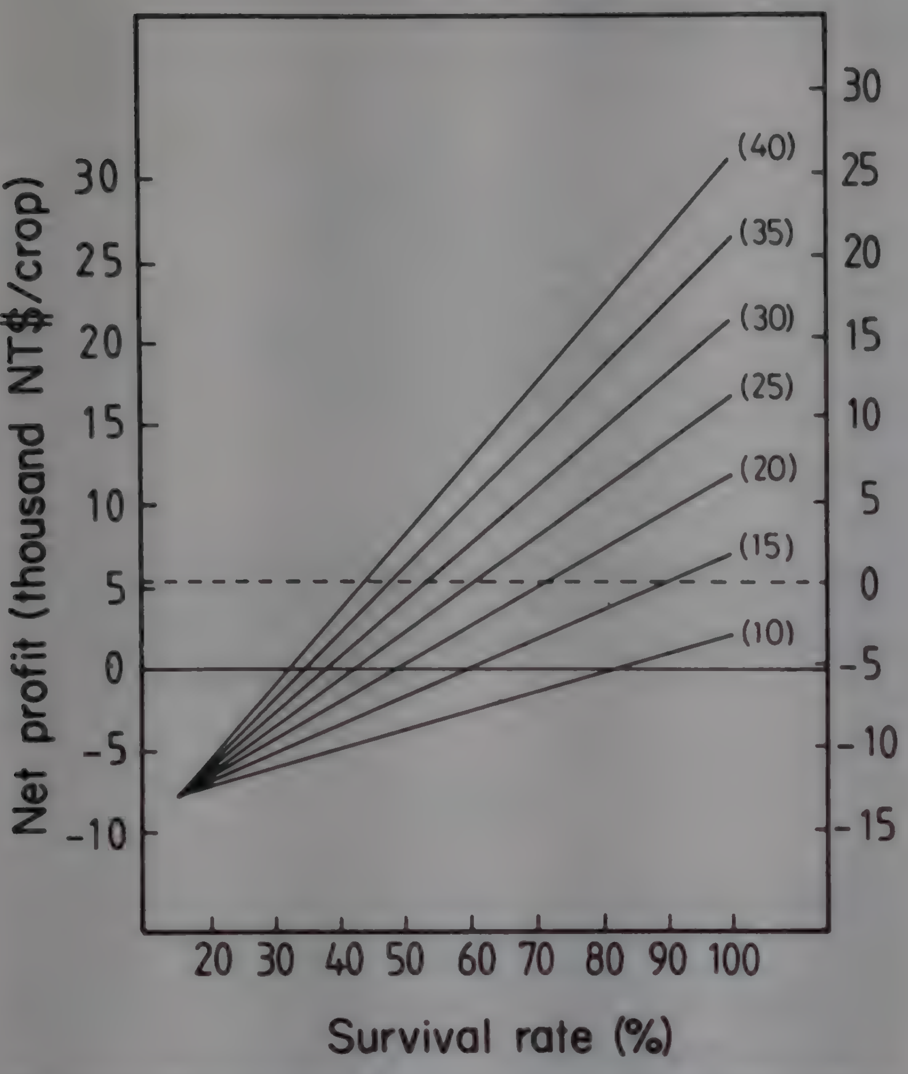

Fig. 4b. Profit projection for semi-intensive culture system (Type B). The stocking densities are indicated in parentheses.

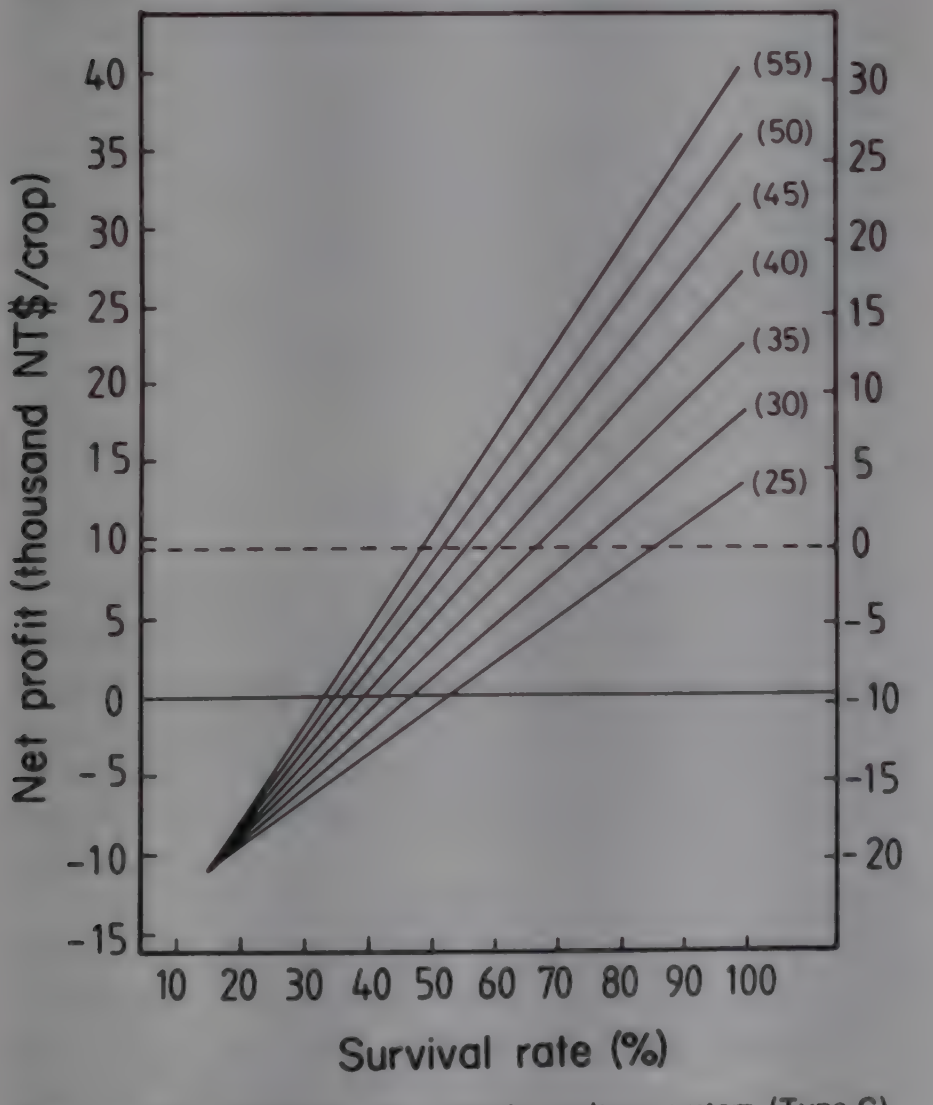

Fig. 4c. Profit projection for intensive culture system (Type C). The stocking densities are indicated in parentheses.

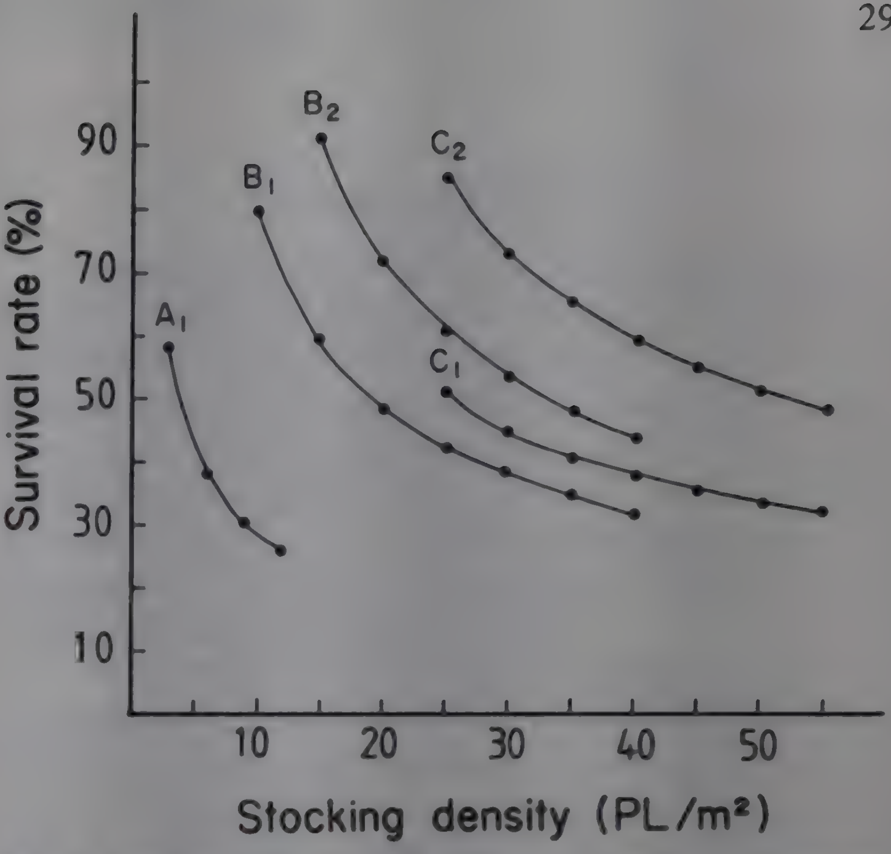

Fig. 5. Break-even lines under the three different culture systems (A, B, C) in Taiwan. 1 represents the cases in which the costs for land, pond and facility construction and manpower are not included in production cost analysis; 2 represents the cases in which the costs for these parameters are included.

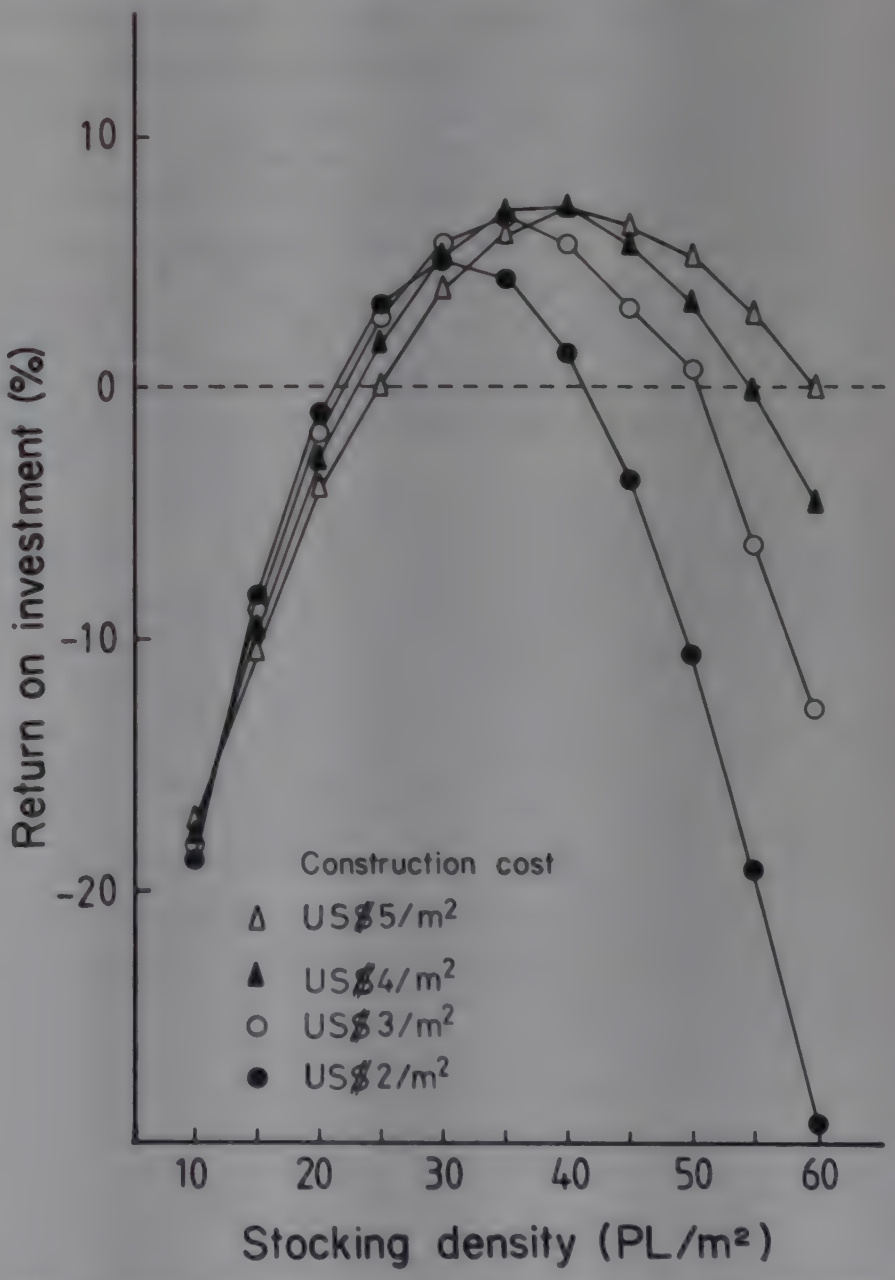

Fig. 6. Return of investment (ROI) under varying investments on the pond and facility constructions.

The Cycle of Nitrogen, Carbon and Phosphorus in an Eel Culture Pond

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CHIBA, K. 1986. The cycle of nitrogen, carbon and phosphorus in an eel culture pond, p. 31-34. *In* J.L. Maclean, L.B. Dizon and L.V. Hosillos (eds.) The First Asian Fisheries Forum. Asian Fisheries Society, Manila, Philippines.

Abstract

To improve eel culture technique, an experiment was carried out in 1981 to identify the cycle of nitrogen, carbon and phosphorus in eel ponds. Eels with total weight of 20 kg were stocked in three concrete ponds each of 32 m^2 and cultured for 16-32 days. Water quality parameters - temperature, pH, dissolved oxygen, inorganic nitrogen and phosphorus - were measured every day. On the initial and final day of the culture periods, soluble and particulate organic nitrogen, carbon and phosphorus were especially analyzed. Fish and sediment were also analyzed for nitrogen, carbon and phosphorus. About 14.3-25.3% of total nitrogen contained in feed was converted by fish. About 75-86% of the nitrogen was therefore transformed into other forms such as soluble matter, particulate matter and organic sediment. The percentages of nitrogen converted from feed to soluble matter, particulate matter and sediments were 15.1-28.6%, 6.7-30.1% and 7.8-13.0%, respectively. Similar results were obtained for carbon and phosphorus. Only 13.3-23.5% of total carbon and 11.1-19.8% of phosphorus in the given feeds were converted into fish; most of these elements were transferred to soluble and particulate matter and to bottom sediment.

Introduction

Generally, it is believed that the environmental conditions in a culture pond are important for fish production because such parameters as the quality of pond water and bottom may have some effect on the physiology of fish, its appetite and growth. However, the relationships between fish production and the environmental conditions are not yet clear.

This study was conducted to clarify the recycling pattern of elements such as carbon, nitrogen and phosphorus in stagnant pond water to know the fundamental recycling pattern of these elements to maintain favorable conditions for fish.

Materials and Methods

Twenty kilograms of eels *(Anguilla japonica)*, averaging about 100 g each, were stocked in two 32-m^2 (8 x 4 m) ponds with a water depth of 0.8 m and cultured from 27 July to 28 August 1981. At 9 a.m. daily, pond water was sampled and feed in excess of 2% body weight/day was offered. The fish were fed *ad libitum* between 9 and 9:30 a.m. Residual feed was weighed to estimate the amount of feed consumed. A 0.25-hp water wheel was installed only in Pond No. 1.

On 11 August 1981, 16 days after commencement of the experiment, almost all the fish in pond No. 2 died due to lack of oxygen. After cleaning the pond another batch of eels weighing 22.25 kg was stocked. To distinguish it from the first trial in Pond No. 2, this experiment was designated as Pond No. 3. In this pond, water was aerated at the rate of 60 l/min.

The following water quality parameters were determined: temperature; pH values (glass electrode method); dissolved oxygen (Winkler method, $NaNO_3$-modified method); total ammonium-nitrogen (indeophenol method); nitrite nitrogen (GR reagent method); nitrite nitrogen (Cu-Cd column method); phosphate phosphorus (Deniges-Atkins method); COD (alkali digestion method); alkalinity (indicator: methyl orange); and transparency (Secchi disk). Water was sampled from the surface and the bottom layers at the center of the pond by siphoning. There was little difference between values of the parameters at the surface and bottom. Only surface values are shown in Figs. 1-3.

To clarify the recycling of carbon, nitrogen and phosphorus, which were added into the ponds as feed, these elements in the feed, eels, sediment, and particulate and soluble matter in the pond water were analyzed at the start and at the end of the experiment. On the final day after sampling water from the ponds, pond water was drained from the surface slowly so as not to stir up the sediment. After the fish had been harvested, all the sediments were collected and weighed. The sediment consisted mainly of precipitated phytoplankton which were utilized by chironomid larvae for constructing their protective tubes. The sediment which still produced oxygen by photosynthesis was not uniformly distributed on the pond bottom, but accumulated at one or two locations in the pond depending on the water current pattern produced by the water wheel or the aeration system. The sediment was thus collected without any difficulty. A portion of the water samples was filtered through Whatman glass filter GF/C to eliminate particulate matter. The eels were homogenized with a homogenizer and a portion of the homogenate analyzed.

Carbon and nitrogen in the feed, fish and sediment were analyzed by using a C-N recorder (Yanagimoto model MT 5000) and carbon in pond water with a C-H-N corder (Yanaginomoto model MT 3), TOC meter (OIC model 0524 B) and the Kjeldhal method. Phosphorus was analyzed by colorimetry after digesting with nitric acid and perchloric acid. Ca, Mg, Na and K were analyzed by the atomic absorption method.

Results and Discussion

The water temperature fluctuated within 23.1-30.7°C. The temperature in Pond No.1 which was provided with a water wheel was always about 1°C lower than in Pond No. 2 which had no water wheel or an aeration system. There was no significant difference in water temperature between Pond No. 1 and Pond No. 3.

Fluctuations of dissolved oxygen in the three ponds are shown in Fig. 1. The biggest fluctuation among the three ponds was observed in Pond No. 3 which had 200-38% air saturation. Although a water wheel was provided in Pond No. 1, super-saturation of dissolved oxygen was observed. However, the variation was smallest among the three ponds. In Pond No. 3 a moderate fluctuation was observed.

Nearly the same trend was observed with pH as with dissolved oxygen. The highest and lowest pH values observed in Pond No. 2 were 9.5 and 7.5, respectively, which was the highest fluctuation among the three ponds. In Pond No. 1, the pH value fluctuated between 9.0 and 7.5, and was almost maintained at about 8. In pond No. 3 pH fluctuated within the same range as in Pond No. 1 and was almost constant at 7.5.

The fluctuations of ammonium nitrogen are shown in Fig. 2. In Pond No. 1, about 10 days from the start of the experiment, ammonium-nitrogen was at a low level but suddenly increased to 7-8 ppm. This was followed by a period of small increases and decreases and eventually a peak of 12 ppm was reached on 20 August 1981 after which the ammonium-nitrogen decreased gradually. In both Pond Nos. 2 and 3 ammonium-nitrogen was also at a low level in the first 10 days after which it increased to 2.7 and 3.2 ppm, respectively.

The concentrations of nitrite nitrogen in Pond Nos. 2 and 3 were always constant at low levels of less than 0.03 ppm and did not fluctuate much. However, in Pond No. 1 towards the end of the experiment, it started to increase and finally settled at 1.9 ppm. The start of the nitrite-nitrogen increase coincided with that of the ammonium-nitrogen decrease from the peak in Pond No.1.

Fluctuations of phosphorus in the three ponds are shown in Fig. 3. At the start of the experiment, the concentrations were maintained at low levels. After 10 days in Pond Nos. 1 and 2 the phosphorus levels started to increase and reached their peaks within 3-4 days. In Pond No. 3 it started to increase 4-5 days after the start of the experiment and the increase was gradual, reaching the peak after 10 days. In Pond Nos. 1 and 3 the start of the increase in phosphorus coincided with that of ammonium-nitrogen but in Pond No. 2 it started 2-3 days after that of ammoium-nitrogen.

The results of eel culture are summarized in Table 2. Weight increase of fish was largest in Pond No. 1, followed by Pond. No. 2 and smallest in Pond No. 3. The longest culture period might explain the largest weight gain in Pond No. 1. The feed conversion efficiency was highest in Pond No. 2 followed by that in Pond No. 1 and lowest in Pond No. 3. Generally, the efficiency of commercial feed is believed to be about 60%. Therefore, the values obtained in Pond Nos. 1 and 2 were almost similar to those obtained by fish culturists.

Comparative proportions of elements in the feed, fish, sediment, and particulate matter and soluble matter in water are shown in Tables 3 and 4.

The C to N values in the feed and the eel were similar but a big difference was observed in the P to N ratio which was higher in the feed than in the eel. Differences in ratios among the three ponds were not large. The sediment resembled phytoplankton, and the ratios of C to N of sediment were almost the same as those of particulate matter. However, ratios of P to N in sediment were quite different from and much higher than those of particulate matter.

When the mineral ratios in each item were compared, differences among the three ponds were small except in particulate matter. All mineral ratios of particulate matter in Pond No. 2 were much lower than those in the two other ponds. Ca concentrations of particulate matter on the final day of the experiment were 2.46, 7.04 and 1.77 ppm in Pond Nos. 1, 2 and 3, respectively. Concentration of other materials, such as Mg, Na and K did not differ among the ponds and were within the range of 0.43-0.76, 2.14-4.24 and 2.59-4.63 ppm in Pond Nos. 1, 2 and 3, respectively. Low mineral ratios of particulate matter in Pond No. 2 could have been due to its high Ca concentration. As water was sampled after mass mortality of fish due to oxygen deficiency in Pond No. 2, the low ratios of P to N, and Mg, Na and K to Ca of particulate matter in Pond No. 2 might have had some relationship with the physiological condition of phytoplankton. Although big differences were not observed between fish and feed in the ratio of C to N, in all items higher values were observed in eel in mineral ratios. Ca contents of fish and feed were 7.82-12.46 and 28.95 mg/g, respectively. The high ratios in fish could have been caused by the lower Ca content in fish. When the mineral ratios in particulate matter and sediment were compared, all the ratios in the sediment were much smaller than in particulate matter. The sediment had high contents of Ca and P in all ponds, so that the calcium phosphate in feed may not have been utilized by fish and accumulated in sediment.

The distribution patterns of C, N and P on the final day of the experiment, as shown in Table 5, differed in each pond. However, in all ponds, C, N, and P were highest in the eel. The patterns were similar in Pond Nos. 1 and 3, but slightly different in Pond No. 2. The percentages of C and N in particulate matter were about 2-3 times higher in Pond No. 2 than those in the others. In contrast, the percentage of P was smaller in Pond No. 2. The ratios of C:N:P were calculated as 27.0:4.2:1, 104.8:20:1 and 28.5:4.5:1 in Pond Nos. 1, 2 and 3, respectively. Average C:N:P ratio in phytoplankton was reported as 20.3-1 (Fleming 1940) while the ratio in freshwater phytoplankton was reported as 20.3-21.7:2.7-3.1:1 (Satomi et al. 1975). Only the ratio in Pond No. 2 was similar to that reported by Fleming (1940) while the others were similar to those reported by Satomi et al. (1975). As mentioned before, in Pond No. 2, after a decreasing tendency in dissolved oxygen was observed, almost all fish died due to oxygen deficiency. This decreasing tendency is caused by the decrease in the photosynthesis ability of phytoplankton. If the C:N:P ratio of Pond No. 2 which differs from those of the others, has some connection with photosynthesis, it is useful to observe this ratio in particulate matter to keep favorable conditions in stagnant-water fish ponds.

The total amounts of C, N and P in the given feed and increments of fish, sediments, particulate matter and soluble matter in water are shown in Table 6. The nitrogen accumulated in fish in all ponds ranged between 14.3 and 25.3% of nitrogen in the given feed but the nitrogen in sediment was rather low, ranging between 7.8 and 10.0%. In all ponds the nitrogen in soluble matter and particulate matter increased considerably. When these nitrogen values were summed up, they were higher than that in the eels. Therefore, most of the nitrogen was accumulated in the pond as soluble and particulate matter. The ratio of nitrogen accumulated in fish to that in the feed was equivalent to the efficiency of nitrogen conversion and this efficiency was fundamentally the same as the feed conversion efficiency. Therefore, in the ponds with high feed conversion efficency, such as Pond Nos. 1 and 2, high accumulations of nitrogen, carbon and phosphorus in eels were observed.

When percentages of nitrogen, carbon and phosphorus in all these trials were summed up, they did not reach 100%. The recoveries ranged from 43.0 to 78.1%, from 51.6 to 93.9% and from 63.4% to 77.7% in carbon, nitrogen and phosphorus, respectively. The low recoveries might be due to denitrification of nitrate in the pond, carbon dioxide exchange between air and pond water and emergence of chironomid larvae.

This study has shown the distribution of elements in the ponds after 30 days of fish culture, which might differ considerably depending on biomass and the length of the culture period. Further studies are needed to clarify the effects of culture period and stocking density. Furthermore, the rate of conversion of elements from one item to another, such as excretion rate of fish, sedimentation rate, sediment decomposition rate, and rate of uptake of elements by phytoplankton, should be measured to elucidate the recycling of elements in the fish pond.

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Table 1. Details of the eel culture experiments.

Pond	Area	Culture period	Total wt fish stocked (kg)	Remarks
1	32 m^2 (8 x 4 m)	27 Jul-27 Aug 1981	20.00	1/4 HP water wheel
2	32 m^2 (8 x 4 m)	27 Jul-11 Aug 1981	20.00	
3	32 m^2 (8 x 4 m)	11 Aug-28 Aug 1981	22.56	aeration 60l/min

Table 2. Eel harvest, growth and feed conversion.

Pond	Stocking (kg)	Harvest (kg)	Weight (kg)	Gain (%)	Amount of feed taken (kg)	Feed conversion efficiency (%)
1	20.00 (227 ind.)	31.20	11.20	56.0	18.23	61.2
2	20.00 (234 ind.)	25.56	5.56	27.8	8.30	66.8
3	20.56 (168 ind.)	23.00	2.45	6.50	6.50	37.6

Table 3. The ratios of C and P to N in feed, eel, sediment, particulate matter and soluble matter in pond water.

	Pond					
	1		2		3	
Item	C/N	P/N	C/N	P/N	C/N	P/N
Feed	5.31	0.29	5.31	0.29	5.31	0.29
Eel	5.14	0.26	4.94	0.23	4.94	0.23
Sediment	6.26	1.87	6.69	1.07	6.61	1.16
Perticulate matter	6.47	0.24	5.24	0.05	6.26	0.22
Soluble matter	1.23	0.06	1.75	0.07	1.18	0.04

Table 4. Ratios of Mg, Na and K to Ca in feed, eel, sediment, particulate matter and soluble matter in pond water.

Item	Pond 1 Mg/Ca	Pond 1 Na/Ca	Pond 1 K/Ca	Pond 2 Mg/Ca	Pond 2 Na/Ca	Pond 2 K/Ca	Pond 3 Mg/Ca	Pond 3 Na/Ca	Pond 3 K/Ca
Feed	0.065	0.227	0.130	0.054	0.226	0.133	0.053	0.229	0.133
Eel	0.087	0.238	1.130	0.098	0.328	0.689	0.133	0.340	0.717
Sediment	0.031	0.048	0.024	0.036	0.056	0.025	0.036	0.055	0.027
Particulate matter	0.206	0.882	1.412	0.108	0.598	0.655	0.245	1.204	1.449
Soluble matter	0.082	1.348	0.051	0.080	1.320	0.040	0.082	1.312	0.041

Table 5. Distribution of C, N and P on the final day of experiment. Quantities are in grams; expressed as percentages in brackets.

Item	Pond 1 N	Pond 1 C	Pond 1 P	Pond 2 N	Pond 2 C	Pond 2 P	Pond 3 N	Pond 3 C	Pond 3 P
Eel	864 (65.2)	4,352 (73.4)	222 (48.6)	726 (62.8)	3,588 (66.5)	165 (60.7)	653 (72.2)	3,229 (78.9)	148 (66.0)
Sediment	107 (8.2)	670 (11.3)	200 (43.8)	81 (7.0)	542 (10.0)	87 (32.0)	49 (5.4)	319 (7.8)	57 (25.4)
Particulate matter	92 (7.1)	595 (10.0)	23 (4.9)	188 (16.3)	986 (18.3)	10 (3.5)	60 (6.6)	376 (9.2)	13 (5.8)
Soluble matter	253 (19.5)	312 (5.3)	12 (2.6)	161 (13.9)	281 (5.2)	11 (3.9)	142 (15.7)	168 (4.1)	6 (2.8)
Total	1,298 (100)	5,929 (100)	457 (100)	1,156 (100)	5,397 (100)	272 (100)	904 (100)	4,092 (100)	224 (100)

Table 6. Total amount of C, N and P in feed, eel, sediment, particulate matter and soluble matter in ponds. Quantities are in grams; expressed as percentages of feed in brackets.

Item	Pond 1 N	Pond 1 C	Pond 1 P	Pond 2 N	Pond 2 C	Pond 2 P	Pond 3 N	Pond 3 C	Pond 3 P
Feed	1,375 (100)	7,300 (100)	4,040 (100)	624 (100)	3,315 (100)	183 (100)	489 (100)	2,596 (100)	144 (100)
Eel	304 (20.1)	1,562 (21.4)	80 (19.8)	158 (25.3)	780 (23.5)	36 (19.7)	70 (14.3)	344 (13.3)	16 (11.1)
Sediment	107 (7.8)	670 (9.2)	200 (49.5)	81 (13.0)	542 (16.4)	87 (47.5)	49 (10.0)	319 (12.3)	57 (39.6)
Particulate matter	92 (6.7)	595 (8.2)	22 (5.4)	188 (30.1)	986 (29.7)	9 (4.9)	60 (12.3)	376 (14.5)	12 (8.6)
Soluble matter	207 (15.1)	312 (4.3)	12 (2.9)	159 (25.5)	281 (8.5)	10 (5.5)	140 (28.6)	168 (6.5)	6 (4.1)
Total	710 (51.6)	3,139 (43.0)	314 (77.6)	586 (93.9)	2,589 (78.1)	152 (77.7)	319 (65.2)	1,207 (46.5)	91 (63.4)

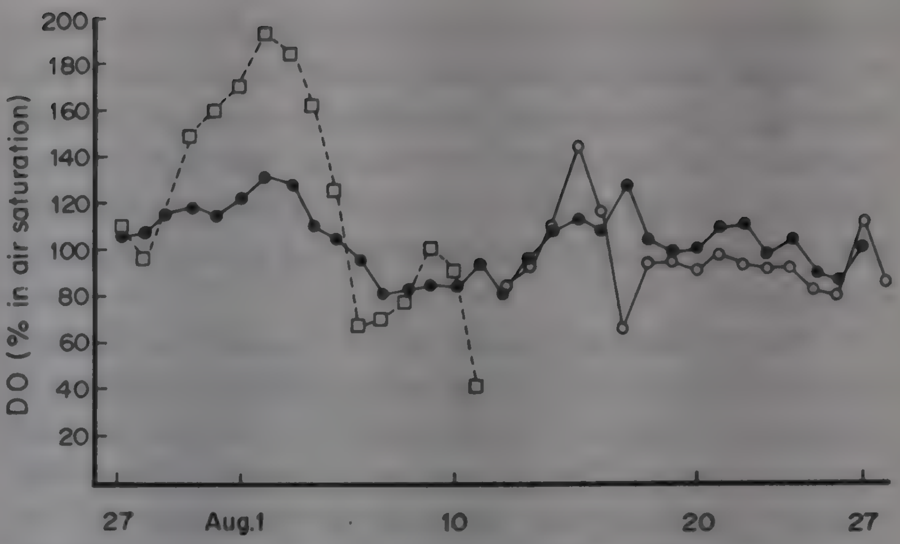

Fig. 1. Fluctuation of dissolved oxygen in the surface layer of pond water. ● — Pond No. 1; □ — Pond No. 2; ○ — Pond No. 3.

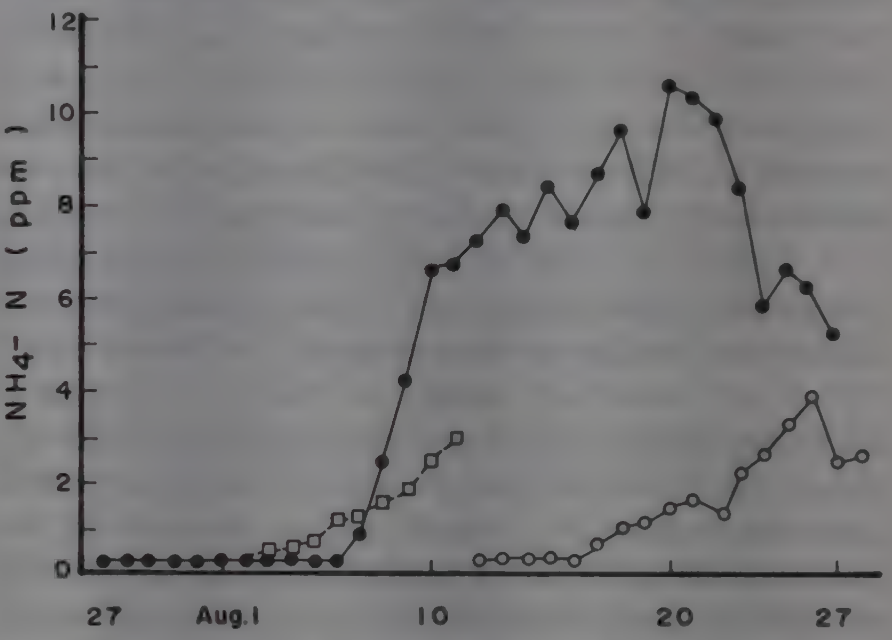

Fig. 2. Fluctuation of ammonium-nitrogen in the surface layer of pond water. ● — Pond No. 1; □ — Pond No. 2; ○ — Pond No. 3.

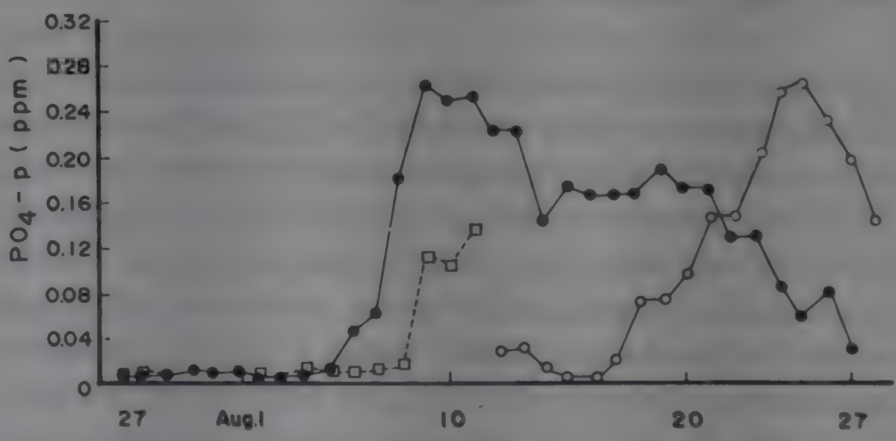

Fig. 3. Fluctuation of phospate phosphorus in the surface layer of pond water. ● — Pond No. 1; □ — Pond No. 2; ○ — Pond No. 3.

The Use of Steel Slags as Substrates in Tilapia *(Oreochromis niloticus)* Rearing

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Abstract

A study was conducted by China Steel Corporation to find out the effects of using slags as substrates in rearing tilapia *(Oreochromis niloticus)* in aquaria. Twelve aquaria were randomly assigned to six substrate treatments: blast furnace slag (BF), converter slag (BOF), granulated slag (GS), concrete (CON), dirt (DT) and control (BNK, glass bottom). Each treatment was duplicated. Tilapia averaging 10 g were stocked in aquaria at 27 fish/m^2 reared for 39 weeks. Water analysis showed Mn^{2+} concentrations in slag treatments were higher than the other three for eight weeks and Fe^{3+} reached a peak of 320 µg/l on the second week. All three slag treatments had higher hardness in water for 12 weeks. Ammonia levels in GS, DT and BNK were consistently higher than those in CON, BF and BOF. BNK had the highest nitrite concentration most of the time. The growth rates of tilapia (g/week) from various substrates in descending order were: CON (1.200), BF (1.140), BOF (1.050), BNK (0.915), DT (0.870) and GS (0.675). The weekly instantaneous mortalities ($Z \times 10^{-2}$) in descending order were GS (1.275), DT (1.245), BNK (1.195), BOF (1.095), BF (0.790) and CON (0.650). Both the growth and survival rates of tilapia seemed affected by ammonia and nitrite more than by heavy metals. No significant differences in Fe^{3+} and Mn^{2+} in liver, intestine and muscle of the resulted fish were found among treatments.

Introduction

The waste steel slag products generated from China Steel Corporation (CSC) amounted to approximately 800,000 t in 1984, including blast furnace (BF) slag, converter (BOF) slag and water-cooled BF slag (granulated slag, G slag).

Both BF and BOF slags are rock-like and G slag is granulated. Slag contains generally calcined lime (CaO) and silica (SiO_2) as principal constituents. BF slag also contains alumina (Al_2O_3) and manganese oxide (MnO). Granulated slag has a vitreous structure and its chemical composition is basically the same as BF slag. All these constituents are those usually composing the crust of the earth, and their chemical compositions are similar to that of average sedimentary rock and Portland cement.

In the Cha-Tung and Lin-Pe area, approximately 30 km southeast of CSC, the massive pumping of underground water for shrimp farming has caused ground depression of 2-3 m and resulted in difficulty in draining and drying of ponds. It is a common practice of shrimp farmers to routinely pump up underground freshwater and mix it with seawater to fluctuate the salinity between 15 and 20 ppt in shrimp ponds to stimulate shrimp molting and subsequently hasten growth. In further inland freshwater aquaculture, a ground depression of 1-2 m was also an effect of massive pumping of underground water in the adjacent shrimp culture area.

CSC evaluated the feasibility of the use of steel slags as fillers for pond bottoms and studied the effects of its use on the growth and survival of tilapia.

Materials and Methods

The study was conducted in the Department of Aquaculture, National Taiwan College of Marine Science and Technology, from August 1984 to April 1985.

Six substrates were used: BF, BOF and G slag (GS), concrete block (CON), dirt (DT) and blank (BNK) - a treatment with nothing added to the aquaria. Each treatment was duplicated. The substrates were evenly spread out about 5 cm thick in 75 x 30 x 40 cm aquaria. Water was maintained at 25 cm in all aquaria. Tilapia *(O. niloticus)* averaging 10.3 g were stocked at six per aquarium (27/m^2). Water temperature ranged from 19 to 25°C.

Every week before changing half of the water, 0.5 l was sampled for chemical analysis. Besides NO_3-N, NO_2-N, S^{2-}, hardness and pH, the heavy metals Fe^{3+} and Mn^{2+} were also measured. Every two weeks fish were sampled and weighed. Tilapia were fed a commercial feed at 3% average body weight at dawn and dusk. Simple linear regression equation $Y = B_0 + B_1X$, where Y is the fish weight in g, X the week, B_1 the growth rate in g/week, was used to show the growth condition. During daily feeding and checking, the dead fish were replaced with similar sized fish to maintain the original stocking density as the growth of tilapia could be density-dependent

(Chen and Prowse 1964; Balarin and Hatton 1979). The weekly instantaneous mortality was calculated by the equation $S_t = S_{t-1} \times e^{-z \cdot t}$ (Heinrich 1981) where S_t and S_{t-1} were the survival rates at t-th week, Z the weekly instantaneous mortality. $S_t = 1$ - (total number of dead fish up to the t-th period / total number of fish stocked up to the t-th period).

At the end of the experiment, two tilapia in each aquarium were sampled to analyze Fe^{3+} and Mn^{2+} content in liver, intestine and muscle by standard methods (American Public Health Association 1981). One-way analysis of variance was conducted to test the differences in growth and survival rate of tilapia and Fe^{3+} and Mn^{2+} content in liver, intestine and muscle of the tilapia among treatments.

Results and Discussion

Manganese. Before the eighth week the average Mn^{2+} concentrations in the slag treatments BOF, GS and BF were all higher than the other three treatments, CON, DT and BNK (Fig. 1). However, after the eighth week, Mn^{2+} concentration in all six treatments were of no marked difference and were lower than 100 μg/l; therefore no analyses of Mn^{2+} were conducted after the 16th week. The Mn^{2+} concentration in BOF remained the highest among all treatments before the eighth week and reached 635 μg/l on the second week but steadily decreased to 74 μg/l on the eighth week. At the start of the experiment, the Mn^{2+} concentration in GS (145 μg/l) was lower only than that in BOF and continued to decrease. The Mn^{2+} concentrations from seven samplings during 16 weeks was lowest in BNK (77.57 μg/l) and with the least variation (S.D. = 6.4 μg/l). Since BF, BOF and GS slag consisted of higher levels of manganese than CON, DT and BNK of no substrate, it is conceivable that the water over the slags contained higher concentrations of Mn^{2+}. When the substrates were just flooded, GS, basically with the same chemical composition as BOF, released more Mn^{2+} than BOF did, for GS had larger granule surfaces in contact with the water. After the eighth week, the treatments with substrate had exhausted their manganese so that Mn^{2+} concentrations became equalized and stabilized.

Ferric ions. In DT and all three slag treatments, the Fe^{3+} concentration increased to above 200 μg/l on the second week, followed by a steady decline until the eighth week, and stabilized below 110 μg/l thereafter (Fig. 1). The Fe^{3+} concentration in BNK and CON fluctuated only between 56 and 103 μg/l. Before the tenth week (except when just flooded), the water with BF substrate contained the highest Fe^{3+} and its concentration reached a peak of 320 μg/l on the second week. Both the average (159 μg/l) and S.D. (82.8 μg/l) of Fe^{3+} in BF were the highest compared to those in the other treatments; BF slag originally contained the highest amount of iron. Concentrations of Fe^{3+} were the lowest in CON (80.4 ± 10.7 μg/l) and in BNK (74 ± 15 μg/l); CON and BNK treatment had the least input of iron.

Hardness. Before the 12th week water hardness in three slag treatments was higher than in the other treatments because of the high content of CaO and MgO in those slags. There was a general trend of increase in hardness in CON, BNK and DT until the 18th week since excess feed or metabolite from the fish might contribute and accumulate the main source of hardness. Before the 12th week the hardness in BOF was the highest and above 200 mg/l. After the 14th week except on one occasion, hardness in BF was the highest and above 200 mg/l. CON had the lowest average hardness (86.6 mg/l) which was not expected as it contained high CaO. GS had the lowest variation of hardness (S.D. = 21.4 mg/l). Both the average and variation of hardness were highest in BOF (228.2 ± 140.9 mg/l); BOF slag contained the highest CaO.

Ammonia. Ammonia concentration (mg/l) generally increased through time in all treatments (Fig. 2). After the 14th week, ammonia in GS, DT and BNK was consistently higher than that in CON, BF and BOF and was greater than 7.0 mg/l. Average ammonia in descending order was BNK (3.9), GS (3.6), DT (3.57), BOF (2.47), CON (2.46) and BF (2.34). The S.D. of ammonia was lowest in BF (1.02) followed by BOF (1.08) and CON (1.14).

Nitrite. Nitrite concentration in all treatments increased through time (Fig. 2). BNK had the highest nitrite concentration all the time except during the second and fourth weeks and reached 5.4 mg/l at the end of the experiment. Except during the last two observations in BF, nitrite levels in BF and BOF were lower than 1.0 mg/l. In both the average (347 μg/l) and S.D. (225 μg/l) nitrite in BOF was lower than those of other treatments and those in BNK were the highest (1,728 + 1,657 μg/l).

Sulfide and pH. Throughout the experiment, water was constantly aerated and S^{2-} concentration stayed below 8 μg/l in all treatments. No significant differences of S^{2-} among treatments or particular trend in S^{2-} profile were found. The fluctuation of pH was in the range of 7.0 to 8.5. There were no significant differences in pH among treatments.

Instantaneous weekly mortality ($Z \times 10^{-2}$) was the highest in GS treatment (1.275) followed by DT (1.245), BNK (1.195), BOF (1.095), BF (0.790) and CON (0.650). However, the statistical test showed no significant ($P > 0.05$) differences in mortality among treatments.

Mortality and growth. The difference in mortality among treatments is hardly explained by heavy metal alone. Although Mn^{2+} in BOF treatments and Fe^{3+} in BF had been higher than those of the other treatments, no apparent effects on tilapia mortality was revealed. Tilapia can tolerate high concentrations of Mn^{2+} and Fe^{3+}. Sublethal effects of heavy metals on fishes were studied by Chen et al. (1980), Wai and Liu (1982) and Wai et al. (1984). Chen et al. (1980) stated that *Oreochromis niloticus* was more resistant to heavy metals than mirror carp, scale carp and grass carp when tested with heavy metals, Cd^{2+}, Hg^{2+}, Cu^{2+}, Cr^{6+}, Fe^{3+} and Mn^{2+}. Tilapia was also most tolerant to Fe^{3+} (TLm 24 hours = 9-15 mg/l) and Mn^{2+} (TLm 48 hours = 750 mg/l). Wai and Liu (1982) found that Mn^{2+} (TLm 24 hours = 34.40 mg/l and TLm 48 hours = 34.38 mg/l) and Fe^{3+} (TLm 24 hours = 140 mg/l and TLm 48 hours = 135 mg/l) were least toxic to tilapia of 1-1.5 cm among 11 heavy metals tested. Compared to Hg^{2+}, Cd^{2+}, Cu^{2+}, Fe^{3+}, Ni^{2+} and Pb^{2+}, Mn^{2+} was least toxic (TLm 24 hours = 33.64 mg/l and TLm 48 hours = 33.58 mg/l) to grass carp fingerlings of average size 3.5-4.0 cm (Wai et al. 1984). In this experiment even the highest Mn^{2+} (635 μg/l) or Fe^{3+} (225 μg/l) was lower than Mn^{2+} or Fe^{3+} TLm 48 hours of tilapia found by Wai and Liu (1982). No long-term effects (eight weeks) of Mn^{2+} and Fe^{3+} concentrations lower than the TLm 48 hours have been studied.

Furthermore, the effects of heavy metal from BF or BOF might be buffered by their higher amount of hardness. As concluded by Chen et al. (1980), the higher the hardness, the lower is the heavy metal toxicity to fish because the solubility of heavy metals is low in water of high hardness from Ca^{2+} and Mg^{2+}.

Lower tilapia survival in treatments of GS, DT and BNK was probably due to higher ammonia and nitrite concentrations. The ammonia concentration in these three treatments was significantly higher than the other three after the 16th week and was higher than 4 mg/l after the 24th week. Tilapia was quite resistant to ammonia. The TLms of ammonia (mg/l) to tilapia of 7.7 cm were 101.86 (24 hours), 54.59 (48 hours), 47.52 (72 hours) and 44.34 (96 hours), which were about one-third higher than eel *(Anguilla japonica)* (Tsay et al. 1982). Tilapia developed extra tolerance for ammonia after acclimation. Redner and Stickney (1979) demonstrated that when *Oreochromis aureus* of 7-9 cm total length were acclimated to sublethal concentration (0.43 to 0.53 mg/l) for 35 days, a concentration of ammonia as high as 3.4 mg/l caused no mortality within 48 hours. In this study over 39 weeks when the tilapia in the GS treatment were acclimated to ammonia up to more than 9.0 mg/l, the overall mortality was 60.82%.

Hwang et al. (1983) found the TLms of sodium nitrite to hybrid tilapia were 16.0, 15.0, 15.5/mg/l during 48, 72 and 96 hours, respectively. Of ten tilapia tested, none died when exposed for 96 hours in water containing 8 mg/l sodium nitrite. The nitrite concentration in this study was no higher than 6 mg/l. Because of constant aeration, the water in DT was more turbid than the other treatments. The second lowest fish survival in DT was suspected due to turbidity at first. However, Balarin and Hatton (1979) stated that tilapia tolerate high turbidities and were rather resistant to pollution by toxic substances, whether organic or inorganic, natural or artificial.

The average growth equations of fish, $Y = B_0 + B_1 X$, where Y is the fish weight in g, X the week, B_1 the growth rate in g/week, in treatments were as follows:

	BNK	DT	GS	CON	BF	BOF
BO	11.842	10.722	12.069	10.935	9.893	11.368
B1 (g/week)	0.915	0.870	0.675	1.240	1.140	1.050

The average growth rates (g/week) of tilapia in substrates of CON, BF and BOF were superior to those in BNK and DT. The poorest growth occurred in GS treatment, which was significantly lower ($P < 0.05$) than the other five treatments. No significant differences in growth were found either between BNK and DT or among CON, BOF and BF, but the growth in rocky substrate CON, BF and BOF were significantly higher than that in BNK and DT.

The concentration of both Mn^{2+} and Fe^{3+} in the fish at the end of the experiment was highest in the intestine followed by liver and muscle (Fig. 3). Since the concentration was in wet base (μg/g wet weight) and the moisture in these tissues was not measured, no statistical tests on the differences of concentrations among tissues were conducted.

The average Mn^{2+} in intestines in descending order was: BOF (3.7), DT (2.9), BF (2.1), GS (1.9), BNK (1.7) and CON (1.4). No significant differences of average Mn^{2+} in intestines among treatments were found. Although Mn^{2+} levels in intestine, liver and muscle of the fish from BOF were higher than those from the other treatments, the order was not exactly parallel in the three tissues. The average Fe^{3+} in instestines in descending order was: BF (12.2), DT (10.3), BOF (9.6), GS (8.4), BNK (7.8) and CON (6.7). The results from statistical tests also showed no significant differences of average Fe^{3+} in intestines among treatments.

Although Mn^{2+} in water from BOF and Fe^{3+} in water from BF were higher than those from other treatments for eight weeks, the accumulation of these heavy metals might have been eliminated during the rest of the experiment resulting in no significant differences of heavy metal content in the fish in the treatments. Hepher (in Pullin 1982) stated that in Israel, both common carp and tilapia remained largely unaffected by heavy metal buildup in heavily manured ponds. The levels of heavy metals in the pond sediments increased with heavy manuring but the fish were present in the ponds for such a short time that heavy metal levels in their tissues always remained well below World Health Organization recommended safe limits. It appeared that tilapia was able not only to resist heavy metals in the environment but also to accumulate insignificant heavy metals in the tissues.

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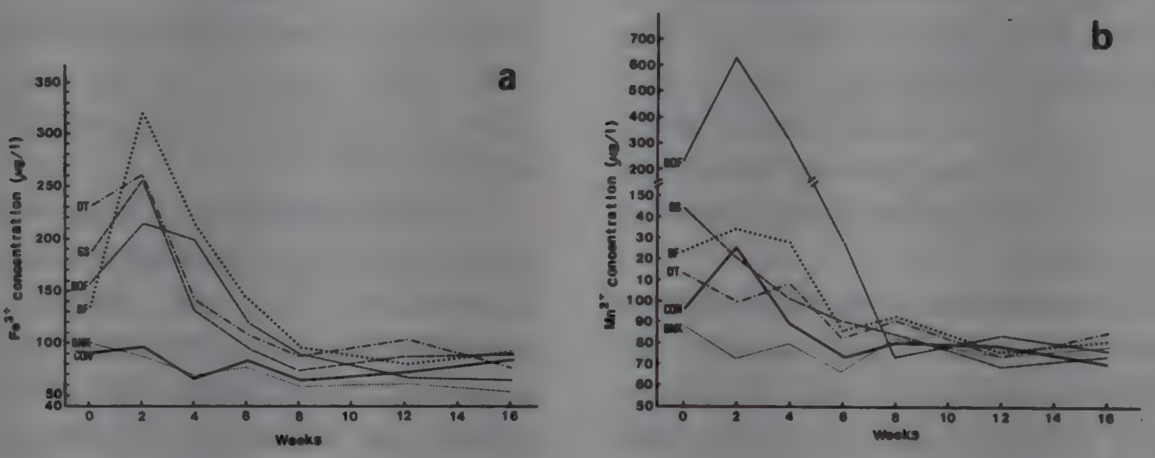

Fig. 1. Concentrations of (a) Fe^{3+} and (b) Mn^{2+} in water cultured with tilapia *Oreochromis niloticus* in aquaria with six substrates: blank (BNK), granulated slag (GS), dirt (DT), blast furnace slag (BF), converter slag (BOF) and concrete (CON) during the first 16 weeks of the rearing period.

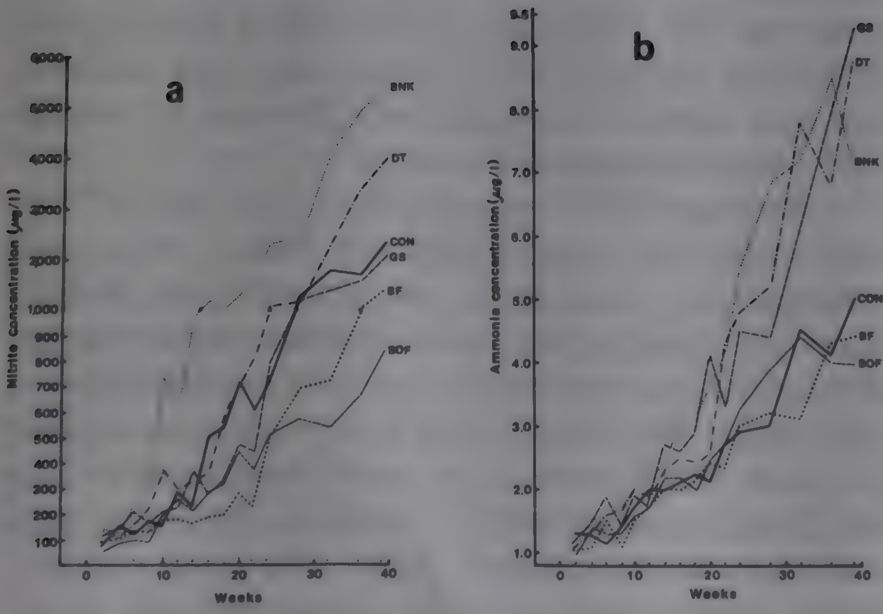

Fig. 2. (a) Nitrite and (b) ammonia concentrations in water cultured with tilapia *Oreochromis niloticus* in aquaria with six substrates: blank (BNK), granulated slag (GS), dirt (DT), blast furnace slag (BF), converter slag (BOF) and concrete (CON) for 39 weeks.

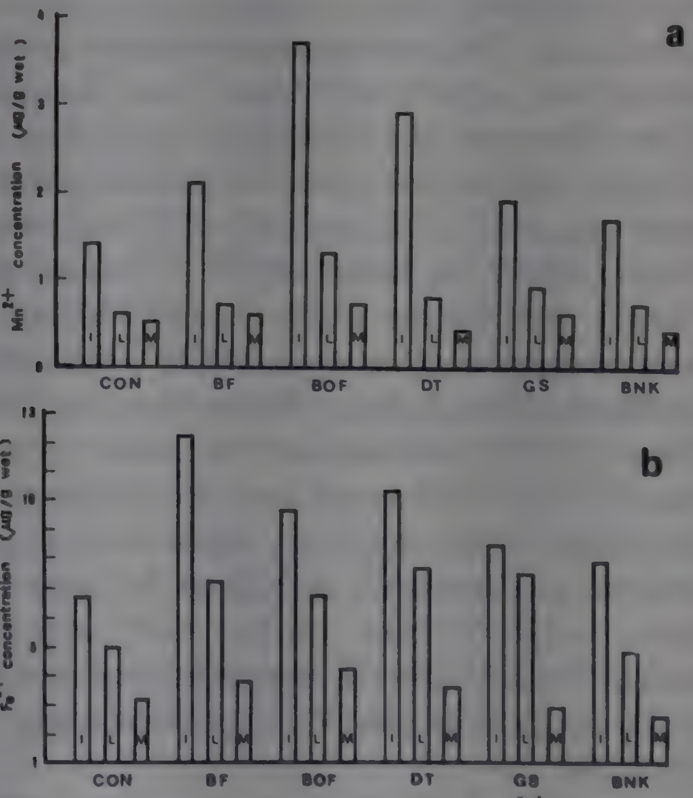

Fig. 3. Concentrations of (a) Mn^{2+} and (b) Fe^{3+} in intestine (I), liver (L), and muscle (M) of tilapia *Oreochromis niloticus* when cultured in aquaria with six substrates: blank (BNK), granulated slag (GS), dirt (DT), blast furnace slag (BF), converter slag (BOF) and concrete (CON) for 39 weeks.

Evaluation of Shrimp *(Penaeus monodon)* Hatchery Methods Practiced by Taiwanese Technicians[1]

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Abstract

From 1974 to 1985 production of shrimp *(Penaeus monodon)* postlarvae (PL) in Taiwan increased from 1.15 million to 3 billion and the number of prawn hatcheries increased from 30 to 1,500. This paper describes the methods in the production of shrimp PL practiced by Taiwanese commercial hatchery technicians. All stages of the hatchery operations, such as maturation, spawning, hatching, larval rearing and algal cultivation, the difficulties encountered in Taiwan shrimp hatcheries and the practical application of these methods outside Taiwan are also discussed.

Introduction

Taiwan produced more than 30,000 t of the shrimp *Penaeus monodon* from aquaculture in 1985 and thus became the number one cultured-shrimp producer in the world (Liao and Chiang 1986). This is due to: (1) successes in hatchery production of fry; (2) improvements in the formulation of compound feed; (3) advances in growout technology; (4) breakthroughs in maturation and larval rearing stages of operation; and (5) penetration of the Japanese market. Unlimited and inexpensive shrimp postlarvae (PL) production in Taiwan greatly stimulated and enhanced the successful expansion of the shrimp culture industry. From 1974 to 1985, stockable *P. monodon* PL production in Taiwan increased from 1.5 million to 3 billion, and the number of hatcheries increased from 30 to 1,500 (Liao and Chiang 1986). During the same period, the PL price dropped from US$0.05 to less than US$0.01 per piece of PL 15-20.

The techniques practiced by Taiwanese hatchery technicians, who usually are not formally trained, are often applied in unsophisticated conditions without the aid of scientific instruments. The methods used by technicians in coping with various hatchery problems are most effective, practical and economical under the circumstances. They are usually adopted rapidly by most hatchery operators in Taiwan.

This paper describes several key methods practiced by Taiwanese technicians and offers a basis for scientific discussions with the hope that these methods can be further explored and improved upon by other hatchery technicians around the world.

Maturation

Initially, only the gravid and ready-to-spawn female shrimp were collected and then allowed to spawn naturally in captivity. Since the 1970s, gravid shrimp have been imported from Southeast Asia to fulfill the needs of the industry. At that time a healthy, large gravid female could fetch a price as high as US$1,800, resulting in substantially higher PL prices during that period. Currently, only non-gravid spawners are imported. Their price ranges from US$40 to US$100 per piece depending upon the season and its market demand.

Taiwanese technicians have been testing the eyestalk ablation method since 1976 (Chen 1977). The success of this procedure was limited until the end of 1982. Through proper manipulation of environmental factors, the broodstock which undergoes unilateral eyestalk ablation could become gravid and spawn repeatedly several times within one to two months. Under normal circumstances, each mature female can spawn more than three times (five to seven times is not unusual) within one month after the removal of the eyestalk. On the average more than

500,000 (up to 1,000,000) nauplii are released during each spawn for which results several key factors are responsible.

Selection of Broodstock

Shrimp with similar physical appearances, but of different origins may respond differently to the maturation technique. It is therefore advisable to run tests on smaller batches before acquiring large stocking quantities from a new source. Experience has shown that shrimp from shallow water coastal areas, or from areas influenced by freshwater runoff give unfavorable results.

The fecundity of shrimp is directly proportional to its size. The preferred body weight of male and female tiger shrimp are 80 to 100 g and 250 to 300 g, respectively. Prawns with a large head-thorax to tail ratio are rejected because they are believed to be older and thus will not reproduce well.

Mature males are identified by the whitish appearance at the base of the last pair of paleopods, indicating fullness in spermatophore. Fertilized females are distinguished by the whitish appearance of spermatophore reflected from below the thelycum.

Shrimp with distinct and bright, yet darkly colored exoskeletons are preferred to ones with dull and/or reddish color. The latter are believed to be unhealthy and less productive. Examination of gill diseases is necessary. Shrimp with rust-colored gills will die easily in captivity, particularly after ablation. Gill diseases have been found to be contagious, thereby threatening the rest of the population.

Environmental Requirements for Maturation

The maturation process is performed in indoor tanks. Heat conservation and total darkness or an extremely dim condition are required for the maturation process. The tank sizes preferred by most technicians are between 20 and 30 m^2 and 1.5 and 2 m in depth, rectangular, concrete and coated with gray epoxy paint. The water level in maturation tanks should be maintained at 30 to 50 cm.

Water is pumped from wells constructed on sandy beaches or in tidal zones less than 3 m deep. The well casing is constructed of perforated PVC pipe (7.5-10 cm in diameter). A 200-mesh nylon bag is fitted over the inlet to avoid the intrusion of impurities. A salinity range of 28 to 33 ppt and a water temperature range of 28 to 30^{o}C are considered optimum. Gentle aeration with aquarium type airstones at about 1/4 m^2 is sufficient. Water is exchanged as required, usually in the morning.

Tanks should be thoroughly washed before stocking and also routinely during the holding period. Afterwards they are disinfected with a household chlorine solution or benzalkonium chloride (50% or 80% solution). Shrimp are given a medicated bath after they first arrive and thereafter on a weekly basis or whenever needed. The most commonly used drugs are Furazolidone (20% A.I. at 10-50 ppm), Dimeton (20 ppm), formalin (100-200 ppm), or antibiotics (at the recommended dosage).

Freshly shucked clams, *Meretrix lusoria,* and oysters, *Ostrea* sp., are the primary diet for the broodstock. Clams are preferred over oysters because of their firmer muscle which does not foul the water. Broodstock are fed three times a day at 2-3 pieces of clams per shrimp or as required. Uneaten portions are removed and discarded to prevent fouling.

A stocking density of 3 to 5 shrimp per m^2 and a male/female ratio of 1:1 or 1:2 are considered the optimal conditions for maturation.

Maturation Technique

The most preferred method of ablation is to tightly snap the eyestalk in the mid-section between the eyeball and the stalk base with a flame heated clamping-forceps. The eyestalk is severed by the heat of the forceps and the clamping action. At the same time, the clamping action also seals the cutting point, avoiding loss of hemolymph. Only female shrimp need to be ablated and unilateral ablation is sufficient.

During the maturation period, natural mating behavior is observed in the night on newly-molted shrimp. If the mating behavior is not observed and/or the gravid prawn are found not fertilized, artificial insemination is applied to assure proper fertilization. The detailed method of artificial insemination on *P. monodon* has been described by Lin and Ting (1985).

An ablated female may become gravid as early as three days following ablation. The peak production of gravid females is between one and three weeks after ablation, although they remain productive for about 30-40 days. The broodstock are replaced with a new batch when their condition deteriorates.

Spawning and Hatching

If the optimum spawning and hatching conditions are not properly maintained, the number of nauplii produced will be low. Several procedures are practiced by the Taiwanese technicians to maximize production.

1. *Environmental conditions.* Requirements are the same for maturation, spawning and hatching. The

spawning and hatching rate increased when the water depth was increased to 1.2-1.5 m depending upon the number of spawners held. The water temperature should be preadjusted to 28-30°C.

2. *Selection of gravid spawners.* With an underwater flashlight in the early evening, gravid spawners can be identified by the dark outline of the ovary in the abdominal portion.

3. *Spawning and hatching.* One spawner per m^2 is the desired density for a spawning tank. After spawning, females remained in the tank until the next morning. Their swimming motion at night stirs the eggs which sink to the tank bottom and facilitates egg hatching. Aeration of the water should remain gentle. The eggs must be stirred from the tank bottom periodically with an oar-like tool made out of a plastic dust pan attached to a PVC pipe. the stirring motion is required hourly or bi-hourly until shortly before hatching.

4. *Collection of the eggs or nauplii.* If the egg collection method is desired, it is usually done in the morning following spawning. Aeration is first turned off and the eggs are allowed to settle to the bottom of the tank. The tank bottom is then drained and the water is passed successively through smaller-mesh filters designed to remove waste materials. The eggs are then rinsed in fresh seawater, transferred to a small holding tank to allow hatching and their number estimated by aliquot sampling.

If the nauplii transfer method is used, it takes place during the nauplii III to V substages. The aeration is turned off and the nauplii are concentrated near the surface. Nonviable eggs and unhealthy nauplii settle to the tank bottom. Water is then drained from some distance off the bottom into a 150-mesh net pan or *hapa* (100 x 60 x 100 cm). The nauplii are then removed from the *hapa* with a hand net and transferred to a holding tank for population sampling, and then to the larval rearing tank.

Algal Culture

A locally available diatom, *Skeletonema* sp., from the eutrophicated Kaohsiung Harbor is collected by a plankton net for the algae stock culture. This species is used almost exclusively for the entire shrimp hatchery industry in Taiwan.

The morphological characteristics of this species of algae have evolved the unique larval rearing method used by Taiwan technicians. *Skeletonema* cells form a long chain and can be made available to the larvae, as required, in the concentrated form by collection through a 200-mesh nylon bag. The other algae species cannot be collected by this convenient method. If the algae is provided with a large quantity of culture media, water chemistry of the larval tank will be affected adversely, placing stress on the larvae.

Algal culture tanks are 5 to 10 t (1.5-m depth) in capacity, concrete, painted with epoxy, and placed outdoors. Technicians have found that the larger the culture tank, the longer the algae may be maintained at the stationary phase. Agricultural fertilizer, potassium chloride (60% A.I.), calcium hyperphosphate (18% A.I.) and urea (40% A.I.) are used at an approximate rate of 8 ppm each. Ferric chloride may also be added at 0.1 ppm. The algal population is maintained by monitoring the culture density and adjusting rates of harvest, dilution and fertilization accordingly.

Larval Rearing

The methods for larval rearing may be classified into several types based on: (1) construction (indoor or outdoor tank), (2) lighting (light or dark) and (3) water quality (clear or enriched culture media). Currently, combinations of different types are utilized by all hatcheries depending upon personal preference.

The indoor hatchery basically is identical to the maturation facility previously described. The light condition of the indoor type may be adjusted by the area of translucent roofing material installed and/or with the plant nursery type shade cloth hanging over the tank. The outdoor types are similar to the indoor ones. Tanks are covered with corrugated plastic roofing material and vinyl-canvas sheet directly. The advantages of the indoor type are that it is more convenient to monitor the light conditions and easier to maintain the water temperature. In view of initial construction costs, however, is more economical to use the outdoor type, which has also the advantage of disinfection by direct sunlight after each harvest. A dim light condition is preferred by most hatchery technicians. Under dim lighting, larvae distribute evenly throughout the water column. Feed distribution and feed consumption are then more efficient.

In the clear-water method, water is exchanged frequently and the tank bottom is siphoned routinely to remove the solid waste. Larvae feed only on the food material provided. In the enriched-water method, water is rarely exchanged. The waste accumulated in the tank bottom, usually in a thick layer by the end of the culture period, should not be disturbed during the culture period. An ecological balance is maintained between larvae, algae, microorganisms and the colloidal organic particles. The latter three are also consumed by the larvae.

Sufficient aeration is required for larval rearing to keep the food particles and larvae evenly suspended. Airstones are hung approximately 5 cm from the tank bottom by plastic air tubing and are evenly distributed at

1/m^2 throughout the tank. Nauplii are stocked at 100-150/liter. Fresh seawater added gradually to the full depth, from 70% initially, eliminates the difficulty of water exchange at the early larval stages. The water temperature should be pre-adjusted to 30°C before stocking and maintained with an automatic water heater apparatus throughout the culture period. The pH value should be monitored continuously and kept steady between 7.7 and 8.3. The water should be exchanged if the pH falls outside this range, otherwise mortality may occur.

Feed is provided in small amounts frequently (6 to 8 times/day). Algae are provided throughout the larval rearing period. For Zoea I and II stages, feed is applied lightly. For the Zoea III stage, sufficient algae must be provided, based on a visual judgement of the algae density and the length of fecal strand created by the larvae. Feeding is provided by spreading concentrated algae evenly over the water surface. Other algae species, such as *Tetraselmis* and *Chaetoceros* are also utilized by some hatcheries.

Artemia nauplii are the food source for the PL stages. They are provided from the Mysis II or III substages to the early PL. At each feeding, only 3 or 4 artemia are provided for each larva. More are given when the first batch is completely consumed. Selection of the brand of artemia cyst is important. Hatchability and essential fatty acid content should be used as the criteria in selecting the brand.

Several types of artificial feed are available. The most commonly used are freeze-dried or spray-dried algae *(Spirulina* sp.*)*, artemia flakes, granular conventional formulated feed, microencapsulated diet and the locally developed specialty diet. All of them are used in all stages of larval culture. For most of the above, feeding is provided by mixing the feed in a bucket of water and then spreading it evenly over the tank or broadcasting it directly over the water surface as required.

The locally-developed specialty diet is unique invention. It is manufactured from several different high protein ingredients, such as squid heads and organs from processing, by-product from soy sauce manufacture, fishmeal and vitamins. Ingredients are processed by heating and fermentation and finally dried into black, semi-moist pellets. Artemia flakes are usually fed simultaneously to the larvae with this product which contains more than 50% protein and effectively increases the survival rate, stimulates feeding behavior and reduces the use of algae and artemia.

Tanks should be thoroughly cleaned and disinfected. A prolonged medicated bath with Furazolidone (20% A.I.) at 10-25 ppm is the popular precaution treatment for all larval stages. The most common diseases in the early larval stages are caused by *Vibrio* spp. and *Leginidium* sp. In the PL stage, protozoan disease caused by *Epistylis* sp. are the most frequently observed (Liao 1985). Unhealthy zoea and mysis larvae may be easily identified by their turning white, loss of vigor, lack of appetite and ultimately death. For the PL stages, unhealthy PL often appear bright red in body pigment, develop forked antennul plate and undergo incomplete molting.

When the larvae reach the PL-10 to PL-12 stages, they are transferred either to the outdoor nursery tank or directly to the grow-out pond. The water is first lowered to 1/3 capacity through the use of a screened siphoning apparatus. At that depth, the stand pipe is pulled and the PL are collected in the net pan. PL are netted into a 12-l bucket in high density from which PL are distributed evenly into a series of 30-l plastic flat 50-cm pans. An experienced person can distribute PL evenly among the pans and can estimate the number of PL in each pan accurately. After the buyer and the supplier have agreed on the total number of PL, each pan is poured into a plastic bag inflated with oxygen and transported by truck to the destination. For long-distance shipping, a styrofoam box is used to hold the bag to maintain the water temperature.

Discussion

In order to gain total control over the entire life cycle of *P. monodon*, research efforts have concentrated on producing in-house broodstock. Two methods have met with initial success. One is the sea ranching method, where pond-cultured animals are released to the sea and allowed to mature in the wild. After a few months, they are retrieved by trawling in the area where they were released. The average return rate is 15% with a record high of 35% (unpublished data). The second method is through pond rearing in which males and females mature in about 6 and 8 months, respectively. By using eyestalk ablation and artificial insemination techniques on these pond-reared shrimp, successful spawning may be obtained. The best results have been obtained on broodstock shrimp that are over a year old. To totally use the in-house broodstock, instead of wild ones, further research in nutritional requirements of the broodstock is necessary.

Although large numbers of PL are produced, the survival rate for the larval rearing remains inconsistent. Total mortality, which could occur at any stage, is not uncommon. Continuous research work is required to identify the sources of disease and their prevention and treatment methods.

Taiwan is a major source of technical assistance for shrimp culture industry development. The hatchery methods developed in Taiwan, however, are specifically derived from local conditions and must be applied elsewhere with caution.

For example, the algae used in larval rearing, *Skeletonema*, is found in dense concentrations in Kaohsiung Harbor, but is difficult to find outside this area. Other diatoms and green algae are available, but the methods used in harvesting and feeding in concentrated form cannot be applied. Algae must be made available to larvae, either through cultivation with the larvae in the same tank or fed to the larvae in large volumes of culture media. The first method must be carried out under light condition, which contrasts greatly with the Taiwanese dark method. The latter approach requires large volumes of water which must be exchanged daily. Again, this contradicts the Taiwanese method of no water exchange. When applying the Taiwanese hatchery methods, one must keep these factors in mind. Contribution B No. 40 from the Tungkang Marine Laboratory.

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Possible Significance of Rearing Conditions of Ovigerous *Macrobrachium rosenbergii* (de Man)

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Abstract

The eggs of *Macrobrachium rosenbergii* incubated at suboptimal temperature (25°C) hatched out in two batches in two consecutive days. The resulting zoea larvae were reared under combinations of two salinities (13.6 and 20.4 ppt) and five temperatures (22, 25, 28, 31 and 34°C) to postlarval stages. Although there were no differences in morphological development of the larvae, both batches showed conspicuous variations in survival rate and adaptation range to temperature. The percentage that metamorphosed was higher in the larvae born on the second day (93.8%) when reared at optimal temperature (28°C). The larvae born on the first day metamorphosed even at 22°C at the rate of 17.5% while the larvae born on the second day did not at all. The median period of each larval stage was almost the same for both batches except when the larvae were reared at the suboptimal temperature of 25°C. In this case the larvae born on the first day developed faster (metamorphosed 3.7 days earlier) than the other batch. These differences are thought to depend on mobilization patterns of the yolk reserve during embryonic development. These results stress the importance of effective rearing methods of ovigerous females in prawn aquaculture.

Introduction

The larvae of several decapod crustaceans are well known to hatch in just several days (Pandian 1970b; Balasundaram and Pandian 1982). The genus *Macrobrachium* is not an exception; for instance, hatching in *M. idae* extends for two to three consecutive days (Pandian and Katre 1972). Wickins and Beard (1974) reported that in *M. rosenbergii* reared at 28°C ± 1°C, a total of 41% of the broods hatched in two consecutive nights. *M. rosenbergii* reared at 25°C during egg incubation was observed to release the larvae invariably in two consecutive batches. Rearing the developing eggs at a suboptimal temperature brings the possibility that they undergo adaptation to a certain extent (Richard 1978) and, consequently, that performance may be affected as stated by Kinne (1964). This study was designed to elucidate the differences in survival and adaptation to temperature among the larvae. The rearing salinities were chosen to be within the optimal range as reported by Ling (1969) and Uno and Yagi (1980).

Materials and Methods

The larvae of *Macrobrachium rosenbergii* were obtained from Anuenue parental stock. The prawns were reared in aquaria containing 45 l of freshwater at 28°C and fed a diet of chopped short-necked clam *(Ruditapes philippinarum)* meat and commercially available penaeid shrimp pelletized feed.

Immediately after mating, the berried female was allowed to acclimatize to the egg incubation temperature of 25°C by changing the water temperature at the rate of 1°C every 2 hours and was reared in the same conditions until the larvae hatched. Within one hour after hatching the zoeae were transferred to 1-l beakers and allowed to acclimatize to the two experimental salinities (13.6 and 20.4 ppt) changed at the rate of 6.8 ppt every 30 min. at the same temperature as the egg incubation. Tap water and offshore seawater filtered through activated charcoal were mixed to obtain the desired salinities.

After salinity acclimation duplicate samples of 40 zoeae were put into 500-ml culture dishes containing 300 ml of rearing water. The culture dishes were placed in constant temperature water baths and the larvae allowed to acclimatize to the five experimental temperatures (22, 25, 28, 31 and 34°C) with the temperature changed at a rate of 3°C every 2 hours. The same procedures were repeated with the zoeae born the second day. The temperature of the experimental water was checked twice a day and readjusted when necessary. The variation was not greater than 0.5°C throughout the experiment. Illumination was provided continuously at intensity 600-1,000 lx.

The zoea larvae were fed daily with newly-hatched *Artemia salina* nauplii. When approximately 50% of the larvae remaining in a culture dish reached the 9th zoeal stage, the rearing was continued in 1-l glass beakers containing 700 ml water. Aeration prevented the larvae from jumping and adhering to the glass surface. The larvae were counted, development was checked and dead individuals eliminated. Then the larvae were transferred by large bore pipettes to clean culture dishes with new water of the same temperature and salinity conditions. Zoeal stages were recorded following the description by Uno and Kwon (1969).

Results

The larvae were observed daily individually, but no differences in morphology or number of instars were found. The median larval period represents the number of days required for the larvae to attain the subsequent stages. In Fig. 1 the median larval period was plotted for each of the rearing temperatures. As could be expected, the duration of larval development was affected inversely by an increase of temperature. At 28°C and 20.4 ppt, the larvae hatched the first day did not differ from second day-hatched larvae. Their median period of metamorphosis was 23.1 and 23.7 days, respectively. The same pattern was observed for the larvae reared at 31°C which reached the postlarval stage in 19.1 and 20.5 days, respectively.

Larvae reared at 34°C did not survive after the sixth stage and their development period was similar. However, larvae from the first day-hatched batch, reared at the suboptimal temperature of 25°C, developed faster (from the fifth zoeal stage on) than the larvae hatched the second day (Fig. 1). Here the median larval period was 30.2 and 33.9 days, respectively, with a gap of 3.7 days.

The most marked difference appears in Fig. 1 for the larvae reared at the suboptimal temperature of 22°C. The first-day larvae developed to postlarvae in 61.7 days while the second-day larvae succeeded to develop only up to the fourth zoeal stage. This trend was observed also at 13.6 ppt of rearing salinity (Fig. 2). The metamorphosis rate was only 7.5% compared with 17.5% obtained when the larvae were reared at 20.4 ppt (Fig. 3). Nevertheless, the fact that none of the second-day larvae metamorphosed at this temperature stresses the differences between the two batches.

Under the other rearing temperatures (25°C and 31°C) the performance of the first-day larvae was superior to that of the second-day larvae (i.e., the metamorphosis rate was higher (Figs. 2 and 3)). When reared at the optimum temperature of 28°C, however, the opposite pattern was observed. The second-day larvae overcame the first-day batch. With salinity at 13.6 ppt, the metamorphosis rates were 87.5% for the former and 78.75% for the later (Fig. 2) while at salinity 20.4 ppt the metamorphosis was 93.75% and 88.75%, respectively (Fig. 3). The effect of acclimation appears to be very important. There is evidence that it may affect the development period and the survival rate as well.

Discussion

Survival and growth of *Macrobrachium rosenbergii* larvae are limited to varying degrees by temperature and salinity, with temperature playing the more important role. The egg incubation temperature of 25°C seems to have modified the adaptation of the larvae to the various rearing temperatures. Adaptation means adjustments at the functional level that bring about an increased efficiency of performance (Kinne 1964). In crustaceans acclimation to subnormal temperatures generally tends to shift the lower limit downward (Kinne 1964; Silverthorn and Reese 1978) and this seems to be the case for the first-day larvae of this experiment. However, suboptimal conditions during incubation may reduce the vigor of caridean larvae (Wickins 1976; Middaugh 1978).

Adaptation by itself does not explain the differences encountered among first-day larvae and second-day larvae. Besides, specific duration of larval development is not considered to be a directly inheritable factor (Sandifer and Smith 1979). Why are the differences between both batches so strong when the larvae are from the same brood? Several authors have made extensive studies on the yolk utilization dynamics of developing crustacean eggs (Pandian 1970a, 1970b; Balasundaram and Pandian 1982; Pandian and Balasundaram 1982). Pandian (1970a) reported a decrease in yolk utilization efficiency in the eggs of *Homarus gammarus* as embryonic development progresses. When hatching is delayed as in this experiment, 26 days and 27 days of embryonic development, respectively, compared with the normal 20 days (Wickins and Beard 1974), protein metabolism is suppressed and there is a shift to fat metabolism, as reported for *M. idae* (Pandian and Katre 1972) and *H. americanus* (Pandian 1970b).

Although no measurement of this type was performed, the depletion of reserve yolk energy in the fully-developed eggs of the second-day batch explains the reduced ability of these larvae to withstand the adverse conditions of suboptimal rearing temperatures (22 and 25°C).

O'Leary and George (1984) examined biochemical changes on embryos of blue crab *Callinectes sapidus* cultured at low (16°C) and high (26°C) temperatures and found also differences in the yolk utilization patterns. It was also pointed out that another possibility for the origin of these differences may be the physiological condition of the female. In our experiment, the higher metamorphosis rates obtained at 28oC for the second-day larvae (Figs. 2 and 3) could be explained by such physiological differences in females at different temperatures. In commercial hatcheries, *M. rosenbergii* larvae are usually reared at 28oC (Silverthorn and Reese 1978). Therefore, to optimize the survival rates, more attention to the rearing conditions of the females is advisable.

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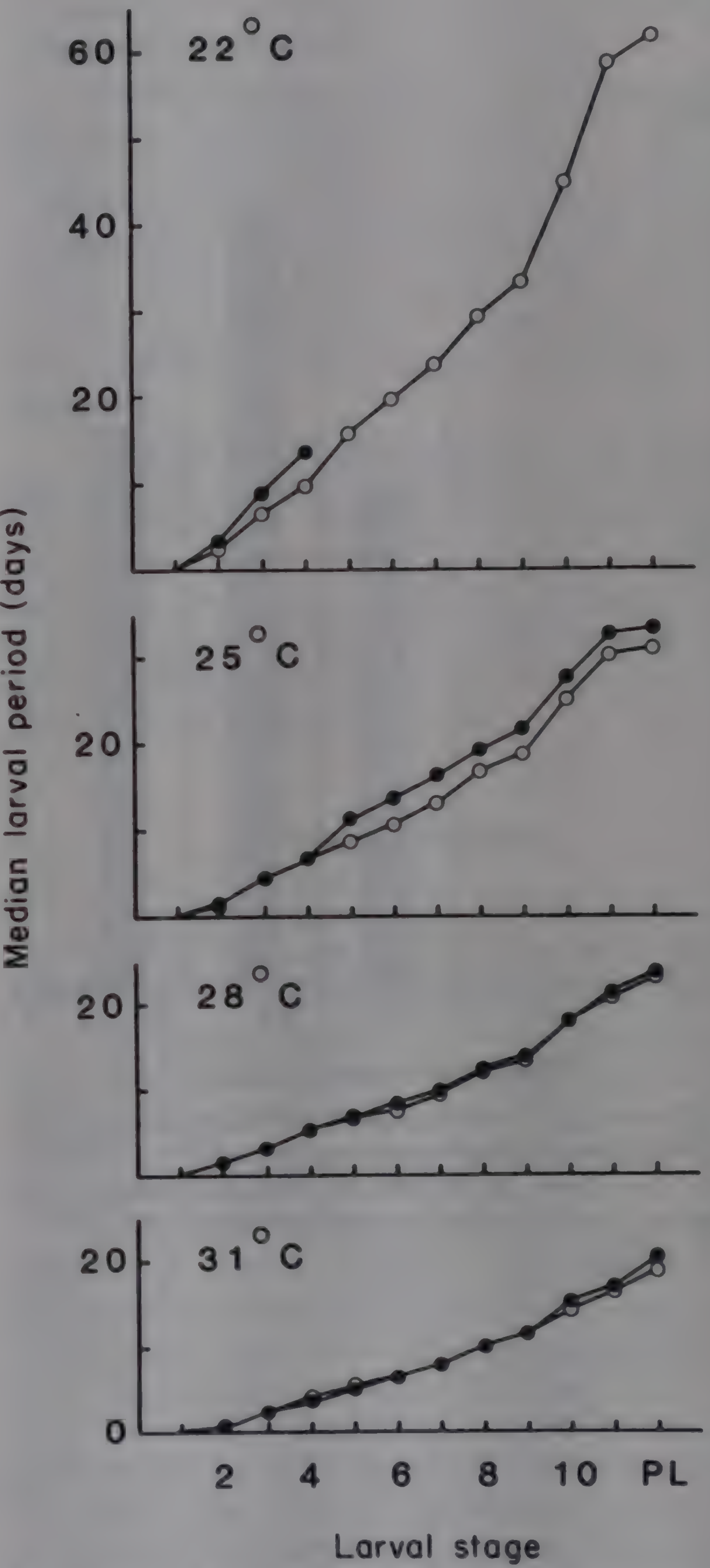

Fig. 1. Median larval period of *Macrobrachium rosenbergii* larvae of the same brood reared at the salinity of 20.4 ppt and four temperatures. Open circles: larvae from the first day-hatched batch. Black circles: larvae from the second day-hatched batch. PL: Postlarval stage.

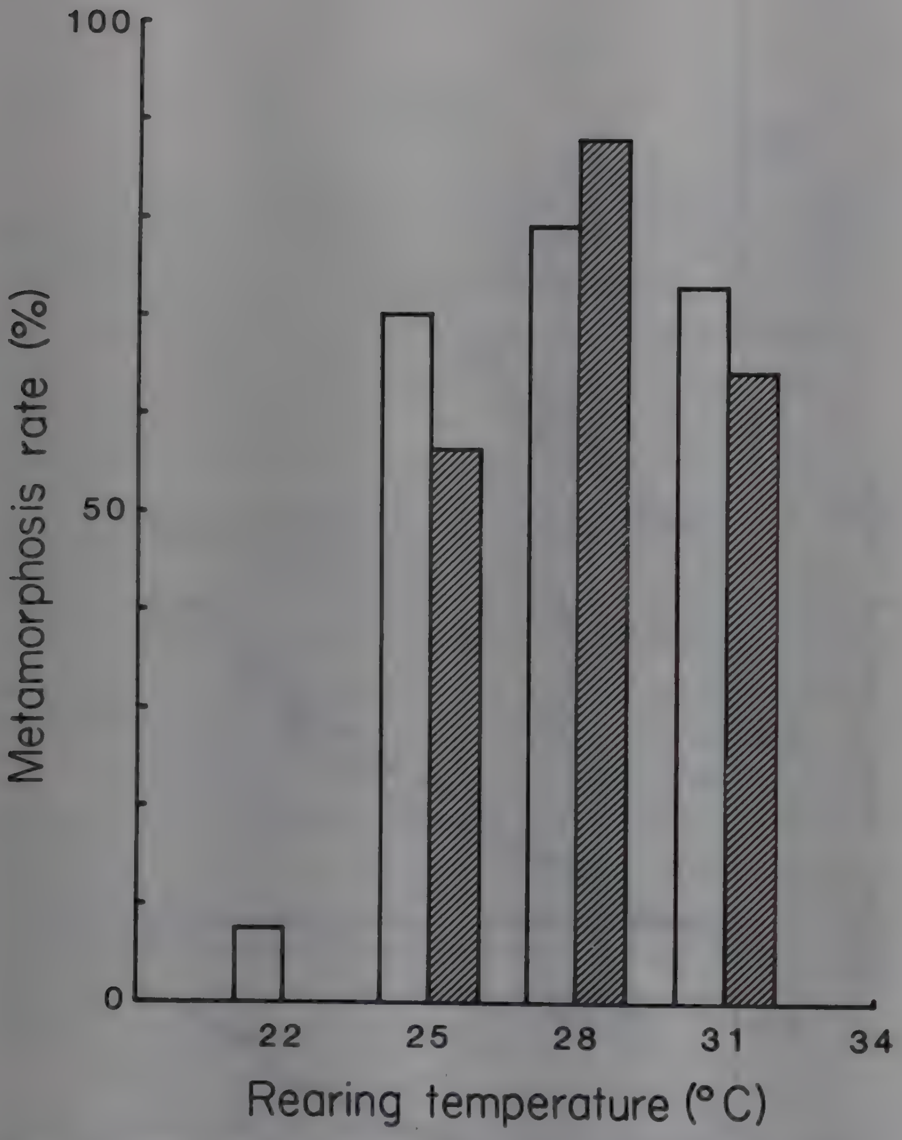

Fig. 2. Percentage of metamorphosis of *Macrobrachium rosenbergii* larvae from the same brood hatched out in two different batches. Open bars: larvae from the first day-hatched batch. Shaded bars: larvae from the second day-hatched batch. Rearing salinity: 13.6 ppt.

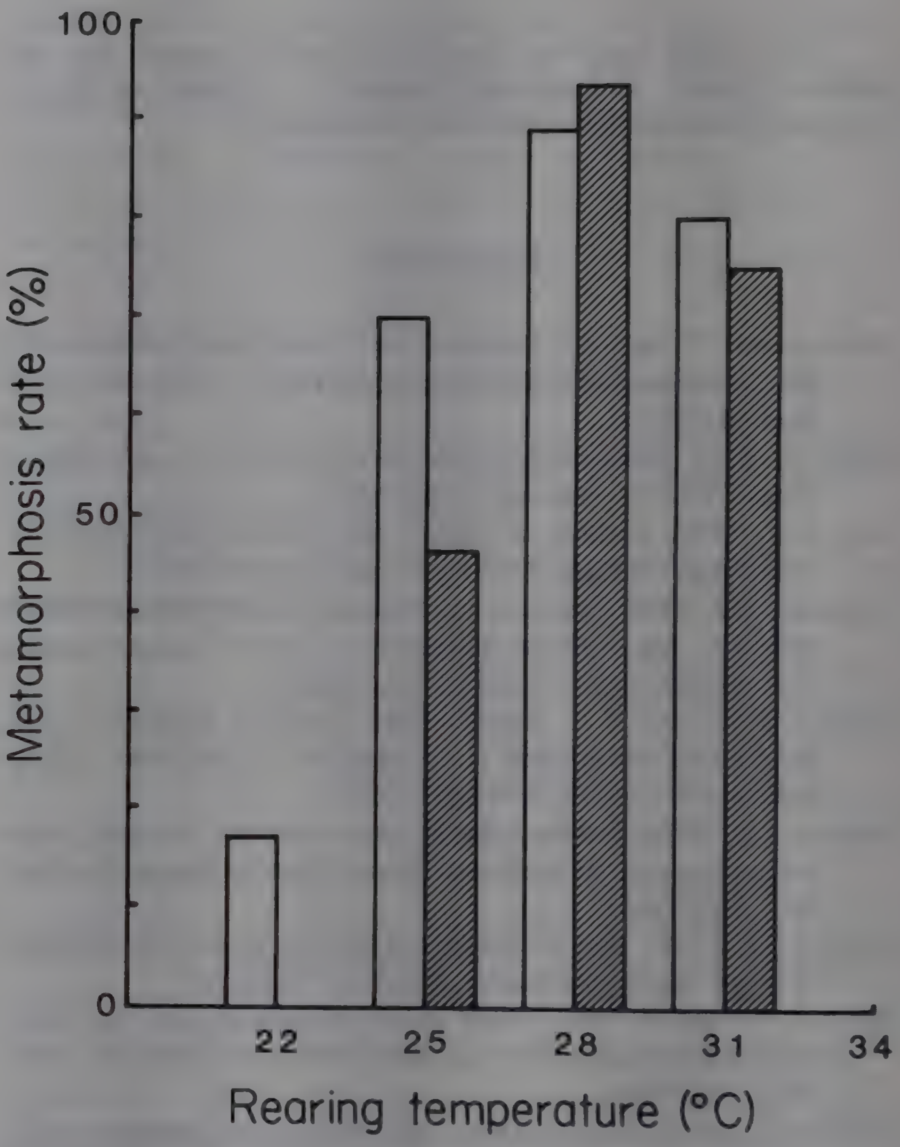

Fig. 3. Percentage of metamorphosis of *Macrobrachium rosenbergii* larvae from the same brood hatched out in two different batches. Open bars: larvae from the first day-hatched batch. Shaded bars: larvae from the second day-hatched batch. Rearing salinity: 20.4 ppt.

Production of Nile Tilapia Fry and Fingerlings in Earthen Ponds at Pila, Laguna, Philippines

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Abstract

A 1.9-ha fish farm was operated for the production of Nile tilapia *(Oreochromis niloticus)* fry and fingerlings in Pila, Laguna, Philippines. The total fry produced by the farm was 2,011,864 in a 159-day production period.

Supplementally-fed breeders were stocked in three earthen ponds at a density of 4/m^2 and sex ratio of three females to one male. Fry yields of 2.4-6.5/m^2/day after 42-43 days were obtained. The first fry appeared 12-14 days from stocking of breeders and fry production peaked at the 32nd-34th day of the breeding cycle.

Fry collected from the breeding ponds were reared in net enclosures for 7 and 12 days at 1,000/m^2 with supplemental feeding. Survival of fry after seven days of rearing was higher than that of fry reared for 12 days.

Fry and postfry stocked in nursery ponds at 170-265/m^2 with supplemental feeding were reared for three weeks to compare for growth and recovery. Better growth and recovery of postfry than those of fry were obtained.

Aside from proper stock manipulation, supplemental feeding and water management, the other factors found essential for high fry/fingerling production were grading of fry/postfry and control of fish predators.

Introduction

The Nile tilapia *(Oreochromis niloticus)*, an introduced food fish in the Philippines, is second only to the native milkfish *(Chanos chanos)* in economic importance for culture. In 1983, a production of over 50,000 t of tilapia was reported in the country. The Philippines has an estimated 1,000 ha of Nile tilapia cages found in Laguna de Bay in addition to about 12,000 ha of freshwater ponds utilized for fish culture (Guerrero 1985).

With the rapid expansion of the tilapia farming industry, production of fingerlings for stocking ponds and cages has increased in recent years. Over a thousand small- and medium-scale tilapia hatcheries are believed to be presently operating in the Philippines with a fingerling production of more than 300 x 10^6 annually.

Several methods are applied for the production of Nile tilapia fingerlings and fry including the use of net enclosures, concrete tanks and earthen ponds. Fry production of Nile tilapia using fine-mesh net enclosures was reported by Guerrero (1977) and Guerrero and Garcia (1963). Yields of 10-240 fry/m^2/month and 70-280 fry/m^2/month were reported by Beveridge (1984) for land-based and water-based hatcheries, respectively, in the Philippines.

This paper reports the production of Nile tilapia fry and fingerlings using earthen ponds in a commercial fish farm operated by the Laguna de Bay Fishpen Development Project of the Laguna Lake Development Authority in Pila, Laguna, Philipines. Observations were made on the breeding cycle of the species. Methods of fry rearing to fingerlings were evaluated. The studies were conducted from January to July 1985.

Materials and Methods

The 1.9-ha fish farm consisted of 19 breeding and 15 nursery ponds with sizes varying from 144 to 535 m^2. Water from a dammed irrigation canal supplied the ponds.

The breeding ponds had a total water surface of 6,621 m^2 and an average water depth of 0.5 m. Inlet pipes of the ponds were screened to prevent entry of wild fish. Drainage of the ponds by gravity was done after each breeding cycle which lasted 30-40 days.

Breeders of Nile tilapia with mean weights of 75 g (males) and 105 g (females) were obtained from government and private hatcheries. The fish were stocked at a sex ratio of one male to three females and density of 4/m^2. Supplemental feeding with a mash ration (60% copra meal, 30% fine rice bran and 10% fishmeal) was given twice a day at the rate of 1-2% of fish body weight per feeding. The schooling fry produced in each pond were collected by means of a fry sweeper twice a day at 6-7 a.m. and 3-4 p.m. The first day when fry were observed from the time that breeders were stocked was noted. Total fry/fingerling count, including those collected at harvest, for each pond was recorded.

The ponds were thoroughly drained, levelled and refilled with water after each breeding/nursery cycle.

Breeders were conditioned for at least one week prior to each cycle.

Fry production in three ponds with sizes of 324, 355 and 415 m^2 was closely monitored to determine the breeding cycle of the species. The number of fry collected in each pond was counted at 10-day intervals (breeding phases) from the initial day of fry collection to a 30-day period.

Two rearing methods, net enclosure and earthen pond, were evaluated for the fry. In the first method, fry were stocked in fine-mesh net enclosures measuring 5 x 2 x 1 m at $1{,}000/m^2$ and fed with a powdered diet (40% copra meal, 30% fine rice bran, 20% *ipil-ipil (Leucaena leucocephala)* leafmeal and 10% fishmeal) at 20% of body weight in four feedings per day. Recovery rates of fry were determined after 7 and 12 days of rearing.

In the other fry rearing method, nursery ponds were stocked with properly graded fry (0.01 g) and post fry (0.05 g) at varying densities ($170\text{-}265/m^2$). Supplemental feeding with a powdered diet (60% copra meal, 20% fine rice bran and 20% *ipil-ipil* leafmeal) was given at 10-15% of fish body weight per day in four feedings. Harvest was done after 21 days of rearing or later when the fingerlings had attained a size of at least 0.5 g. Recovery rates of the young fish in the nursery ponds were measured.

Results and Discussion

The fish farm produced a total of 2,011,864 fry in 159 days (January-July) for a daily production of 1.9 $fry/m^2/day$ (Table 1). There were only 10 days of fry production in January owing to startup operations. Fry production was lowest in June when the irrigation dam broke due to rough weather and resulted in low water supply. The farm's overall fry production is within the range reported by Beveridge (1984) for land-based tilapia hatcheries in the Philippines.

The production of the three breeding ponds ranged from 2.4 to 6.5 $fry/m^2/day$ after 42-43 days from stocking of breeders (Table 2). The first fry appeared 12-14 days from stocking of breeders. Fry production in all ponds was highest at Phase II of the breeding cycle. The production of fry peaked at 32-34 days from stocking of breeders and declined.

The findings of this study slightly differed from those of Guerrero and Guerrero (1985) who reported on the breeding cycle of Nile tilapia in concrete tanks. Fry appeared earlier at 11 days and fry production peaks were reached 17-25 days from stocking of breeders in the latter study. These variations can be attributed to differences in climatic and cultural conditions in the two studies. The breeding period of fish in Guerrero and Guerrero's study took place in July-October while that of the present study was in January-February. Moreover, the tank study used bigger sizes of broodfish (95-140 g) compared to those (75-105 g) in the earthen pond study.

Ambient air temperatures are generally lower during the months of January-February in the Philippines than in other months of the year because of the cold northeasterly winds. Broodfish size is known to affect Nile tilapia fry production among other factors (Silvera 1978).

Table 3 shows a very low survival rate of fry with the longer rearing period which was mainly due to poor water quality in the enclosures and cannibalism of the bigger fry. The fine mesh of net enclosures is easily clogged with organic materials which impede water flow for aeration and waste removal. Crowding of fry for long periods in net enclosures encourages cannibalism.

The recovery and percent of harvestable fingerlings of postfry reared in nursery ponds for three weeks were better than those of fry (Table 4). These indicated that stocking of larger fry in nursery ponds can shorten the rearing period for production of harvest-size fingerlings (0.5 g) and increase recovery rates.

Nursery ponds B, C and D were found to be infested with predaceous fishes such as mudfish *(Channa striata)* and Thai catfish *(Clarias batrachus)* at harvest. Fry appeared to be more vulnerable to predation than postfry.

Aside from proper stock manipulation, supplemental feeding and water management, the other factors found to be essential for high production of fry and fingerlings in earthen ponds were grading of fry/postfry at stocking to minimize cannibalism and effective control of predatory fishes.

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Table 1. Monthly fry production record of fish farm, January to July 1985.

Month	No. of fry	No. of days
January	70,245	10
February	473,040	28
March	353,133	31
April	427,125	30
May	418,188	31
June	82,187	20
July	187,946	9
Total	2,011,864	159

Table 2. Fry production record of three breeding ponds.

Pond no.	Breeding phase	Fry production	% of total fry production	No. of fry/m^2/day
	I	7,100	30.9	2.2
1	II	9,450	41.1	2.9
(324 m^2)	III	6,450	28.0	2.0
		Total 23,000		Mean 2.4
	I	13,370	21.1	3.8
2	II	36,700	56.8	10.3
(355 m^2)	III	13,300	20.1	3.7
		Total 63,370		Mean 5.9
	I	20,200	25.1	4.9
3	II	40,700	50.6	9.8
(415 m^2)	III	19,550	24.3	4.7
		Total 80,450		Mean 6.5

I — First 10-day period after first appearance of fry.
II — Second 10-day period after first appearance of fry.
III — Third 10-day period after first appearance of fry.

Table 3. Survival rates of fry reared in net enclosures.

Rearing period (day)	Net enclosure		% survival
7	1		74
	2		85
		Mean	79.5
12	3		13.5
	4		15.0
		Mean	14.2

Table 4. Comparison of fry and postfry rearing in earthen ponds.

Stage	Nursery pond		Density (no./m^2)	% recovery	% of harvestable fingerlings after 21 days
Fry	A		263	80.1	26.8
	B		178	34.6	15.7
		Mean	220.5	57.3	21.2
Postfry	C		265	74.4	31.8
	D		170.3	70.5	30.4
		Mean	217.6	72.4	31.1

Cultivation of Giant Clams: Beyond the Hatchery

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Abstract

This article describes methods developed over a five-year period for culturing tridacnid clams from seed size to sexual maturity in the natural environment. Design requirements for ocean-based nursery systems are reviewed and various culture options considered. Details are given for one proven method, a bottom-based system of modular nursery cages. To date, 2,000 such units have been constructed and deployed at the Micronesian Mariculure Demonstration Center giant clam farm in Palau.

The basic nursery cage is a 60-cm square, molded plastic tray with an attached lid of plastic mesh. A layer of basalt chips or coral rubble added to the floor of the tray serves as ballast and provides a substrate for byssal attachment by the clams. The units are lightweight, stable, inert in seawater, easy to mass produce and effective in excluding most crustacean, molluscan and teleostean predators. At the same time they allow acceptable levels of light penetration and water exchange.

An analysis is given of material and labor costs, performance in field trials, biofouling and projected lifespan. Guidelines are presented for selecting appropriate tridacnid culture sites in the natural environment and for deploying and maintaining the nursery cages on the sea bottom.

Introduction

During the past decade much of the interest in mariculture of tridacnid clams has focused on problems related to spawning and larval rearing. Publications by Munro and Heslinga (1983), Heslinga et al. (1984), Heslinga and Watson (1985), Heslinga (in press), Heslinga and Fitt (in press) and Munro (in press) provide an entry to the literature on tridacnid biology and culture. In this artcile we address the question of how best to culture giant clams from seed size (2-3 cm) to escape size (10-15 cm) in the natural environment. Escape size is defined as the size range within which immunity from natural predators is reached or closely approached (Heslinga et al. 1984). The objective is to develop a rearing method which maximizes rates of survival and growth and which, to the extent possible, minimizes cost. It seems likely that the degree of success achieved in nursery culture will ultimately determine the economic viability of giant clam farming.

Because giant clams are atypical bivalves, an ocean culture system for these molluscs must depart considerably from conventional methods. Most edible clams burrow completely in soft substrates and communicate with the overlying water via extended siphons. Controlled culture requires exclusion of predators from above and prevention of loss through deep burrowing (Castagna 1984). Tridacnid clams, in contrast, rest upright on hard substrates. The larger species *(Tridacna gigas* and *T. derasa)* attach with byssal threads early in life and do not burrow. During daytime the adductor muscle is relaxed, the valves gape and the hypertrophied mantle is fully exposed to incident sunlight. Symbiotic algal cells residing in the mantle release photosynthetically fixed carbohydrates directly into the host's bloodstream. These photosynthates appear to satisfy a major portion of the clams' nutrition (Trench et al. 1981; Fisher 1985).

Although adult tridacnids are remarkably predator-resistant, the juveniles are vulnerable to a host of common coral reef predators including octupuses, hermit crabs and molluscivorous fishes (Heslinga et al. 1984). In addition, at least two benthic gastropod predators *(Chicoreus ramosus* and *Cymatium muricinum)* are known to attack giant clams in nature by insertion of the proboscis into the byssal opening (Perron et al. 1985). Apart from the need for protection from these and other potential predators, juvenile clams under cultivation require exposure to sunlight, access to a hard substrate for byssal attachment and clean, warm seawater with a minimal silt load and good tidal flushing. Optimal culture temperature appears to be in the range of 23-30°C and optimal salinity is 33-35 ppt, similar to that of oceanic seawater.

Design Conditions and Requirements for Giant Clam Nurseries

Given the above considerations, an ideal giant clam nursery system should meet the following criteria:

1. Exclude swimming and bottom-dwelling predators.

2. Allow good light penetration and unrestricted water flow.
3. Provide a suitable surface for byssal attachment.
4. Allow easy access to the clams by the farmer.
5. Be able to withstand heavy weather.
6. Be inexpensive, lightweight and inert in the marine environment.
7. Require minimal maintenance.
8. Have a moderately long lifespan.

Other features that might be desirable (or necessary) in certain situations include the following:

1. The culture system should not inferfere with coastal navigation or scenic views.
2. It should not damage or permanently alter the natural features of the sea bottom.
3. It should be available commercially at a reasonable price, or if not, be easy to construct with local labor and readily available materials.
4. It should be modular to facilitate construction, deployment and stock inventory, as well as to restrict the movements of pests.
5. It should be replicable on a regional scale.

Although floating rafts have often been proposed for tridacnid culture, this option was not practical for the MMDC nursery. First, we felt that rafts would present an unacceptable hazard to navigation and interfere with the scenic beauty of the culture site in Malakal Harbor; second, rafts would be susceptible to damage by wind, waves and vandals and might require substantial maintenance; and third, the cost per unit of cultivable surface area would undoubtedly be high relative to bottom culture. In addition to heavy-duty mooring hardware, commercial-scale nursery rafts would need substantial floatation material and a solid, relatively stable, horizontal surface for byssal attachment. These are demanding requirements for structures that might approach a hectare or more in size.

Use of the three-dimensional water column for hanging culture, as is practiced commonly with oysters and mussels (Davy and Graham 1982) was also ruled out because giant clams need unobstructed access to sunlight. This requirement will limit any ocean-based culture system to two dimensions.

Because the MMDC tridacnid hatchery lies immediately adjacent to a sandy fringing reef of 3-5 m depth and 1-ha area, the decision was made to use this subtidal bottom as an experimental clam nursery site. Could intertidal areas (which dry out at low tide) also be used for culturing tridacnids? This possibility is being investigated with *Tridacna gigas* in Australia (Lucas, pers. comm.). In this article we confine our discussion to subtidal culture with *T. derasa*.

At the MMDC nursery site, modular trays were chosen as the most promising predator-exclusion device to investigate. Of the commercially available shellfish trays, one design, the Nestier tray (Vanguard Industries, Cincinnati Ohio) was tested for a five-year period *in situ* in Malakal Harbor. The trays were filled with basalt chips and fitted with polyethylene mesh lids to exclude predators. The sample of 100 trays performed satisfactorily, but were far too expensive (US$15 each excluding the lid) to be purchased in large quantity. However, an acceptable substitute for Nestier trays can be constructed from fiberglass mat and polyester resin, at a savings of nearly 70% in cost, including labor and shipping.

While giant clam nursery culture trays may be constructed in a variety of sizes, we recommend units measuring 60 x 60 cm and 5 cm in depth. Trays of this size are easily handled by one person both on land and under water. Moreover, these dimensions permit efficient construction of male molds using standard lumber stock and efficient use of fiberglass mat in standard 97-cm widths.

The tray molds are nailed atop individual tables measuring 70-cm wide by 140-cm long by 86-cm high. The molds are built from 5 x 5 cm frames and a 0.64-cm top.

Materials needed for construction of one tray are listed in Table 1, and basically consist of fiberglass mat, polyester resin, catalyst, a thin plastic mold liner and some very simple tools. The mat is cut to size, placed over the mold and thoroughly saturated with catalyzed resin. Within a few hours the resin is cured and the trays are ready for use.

The technique for making these trays is very straightforward and can be found in any book on fiberglass boatbuilding such as Hankinson (1982). A detailed technical report on making clam trays is available free upon request from the authors.

Lids for the clam trays are constructed of polyethylene Naltex mesh (Nalle Plastics, Austin, Texas) or its equivalent, with a 2.5-cm mesh size. Beginning with a piece of mesh 1 x 1 m, squares of 12 cm are cut from each corner with snipping shears. The corners of the mesh are brought together and laced with 34-kg test monofilament fishing line. The completed lids are attached to trays using two monofilament "hinges" (two holes must be drilled through the lip on one side of each tray). On the opposite side of the hinge a simple latch can be made by passing a piece of brass wire from the mesh to a small hole in the lip of the tray.

It is feasible for one person working alone to construct ten complete giant clam nursery cages (trays plus lids) in an eight-hour work day, assuming that ten molds and all materials are at hand. Lumber for mold construction costs about US$20 per unit.

A complete cost breakdown of materials used for construction of the MMDC type clams is given in Table 1. For each cage of 0.36 m^2 of cultivable area, the cost of materials was \$27.22/m^2 and \$3/m^2 for construction. Deployment of the cages (\$0.55/m^2) and filling with basalt chips required about 20-man hours per cell of 100 units. MMDC's present 2,000 cages offer 720 m^2 of cultivable area and cost approximately $22,000 in 1984.

Performance

The effectiveness of the nursery cages in excluding common tridacnid predators was demonstrated by the results of replicated field experiments conducted in 1984 on the Malakal Harbor fringing reef. In each replicate, a sample of 200 cultured *Tridacna gigas* (mean shell length, 4 cm) was divided into two statistically similar subsamples. One subsample was placed in a nursery tray with mesh lid, the other subsample was placed in an adjacent tray with no lid. Survival of the clams was monitored on a daily basis. The unprotected clams experienced total mortality within a week of release, while the protected clams exhibited 100% survival during a two-week observation period. These results corroborate earlier findings (Heslinga et al. 1984), which showed that unprotected giant clam juveniles in the size ranges tested experienced very heavy mortality in the natural environment, even over a short time period. Specimens protected by nursery cages were essentially immune from predation by crushing fishes, crabs and octupuses.

Because all of the materials used in the production of the nursery cages are inert in seawater, it is reasonable to project a functional lifespan of over 10 years for the units. During a five-year (1982-1986) test period in Malakal Harbor, polyethylene mesh lids showed no obvious signs of deterioration. Fouling of the mesh by filamentous algae was minimal when a few hundred cages were present, being controlled effectively by the grazing activities of scarids, siganids and acanthurids. As the size of the nursery increased, however, algal production appeared to exceed the cropping capability of the resident grazers particularly in deeper water cages. A succession of algal species was evident on the mesh lids, with relatively fine, fragile forms giving way over time to hardier, turflike species. To control algal fouling, the lids are removed and sun-dried every 4-6 months.

Site Selection

The choice of an appropriate ocean-based site for tridacnid nursery culture depends on a number of factors, both environmental and sociological. As in any farming operation, legal access to the site and effective surveillance measures are necessary prerequisites.

Our experience suggests that sheltered, subtidal areas with a good tidal flow and a depth range of 1-5 m are most appropriate. Water temperature should be 23-30°C; salinity, 33-35 ppt; and thriving stony corals should be present in the immediate vicinity. The bottom should be flat or gently sloping and the substrate should be composed of course sand or finger-sized coral rubble, not mud or silt. Care should be taken to avoid areas with high densities of thalassinid shrimp (Suchanek 1985) which may disturb clam trays by ejecting low, volcano-like mounds of sand from their burrows. Mangrove areas and freshwater runoff should be avoided. The site should not be affected by turbulence from breaking surf and should be away from areas where a long fetch can allow buildup of surface swells during storms. The presence of natural populations of thriving tridacnids in the area is by far the best indicator of site suitability. However, strongly byssate species like *Tridacna maxima* and *T. crocea* often occur on seaward reef slopes in high energy surf zones, and these are obviously not appropriate sites for mariculture.

On atolls, sandy subtidal areas behind barrier reefs and leeward sections of lagoon fringing reefs should be appropriate for raising giant clams. On high islands with only fringing reefs, sandy or rubble-strewn areas with appropriate water depth, temperature and salinity should be suitable if sufficiently calm. Seasonal changes should be borne in mind and local advice sought under all circumstances. If any doubt exists about the potential suitability of a site, an assay should be conducted with a small sample of juvenile clams before large numbers are put at risk.

It is worth emphasizing here that the type of subtidal habitat recommended by Doty (1983) for farming the red seaweed *Eucheuma* seems exceptionally well suited for cultivating tridacnid clams. One may readily envision the advantages of *Eucheuma*–tridacnid polyculture system in which the benefits and costs of reef tenure and farm surveillance are shared. The species are complementary too: *Eucheuma* is a fast growing cash crop requiring considerable inputs of time and labor, while giant clams are a relatively slow maturing food crop which demand little care, especially in the grow-out phase.

The nursery trays with attached lids are placed on the bottom at the selected site in rows of 10, with 10 rows making a "cell" of 100 cages. A nursery may contain any number of cells; the MMDC facility in Palau presently contains 20 cells. The rows should be at least 50 cm apart and the cages, about 20 cm apart within the rows. Deployment is best accomplished by two divers from a boat or from shore with scuba being optional depending on depth. As soon as the cages have been arranged on the bottom, about 4 kg of basalt chips or coral rubble are added in a uniform layer to each tray, being poured from a bucket directly through the mesh lid. The chips provide ballast and prevent displacement of the cages under all but extreme conditions.

Planting, Transplanting and Pest Control

When deployment of the cages has been completed, clams are placed inside the densities shown in Table 2. It is generally not desirable to plant clams less than 2-3 cm in length because these may be subject to smothering by sediment or to attack by small crushing predators which enter through the polyethylene mesh.

Following stocking of the trays, routine maintenance consists of pest control, monitoring of growth and survival, and transplanting. If the predatory gastropod *Cymatium muricinum* appears, each nursery tray should be checked twice weekly and any snails and/or dead clams removed (Perron et al. 1985). As a rule of thumb (referring specifically to *Tridacna derasa* and 60 cm trays) the clams should be transplanted at intervals of 5-6 months and thinned to half their former density (see Table 2). The transplants afford the most convenient opportunity to assess growth and survival.

When the cages in a cell have been left temporarily empty, such as after a transplant, it is beneficial to prop open the mesh lids for period of a week or so. This allows predators like wrasses, goatfish, triggerfish and emperors to browse through the basalt chips, consuming any snails or other potential pests before the next cohort of clam seed is planted.

Outplanting

By the time *T. derasa* juveniles reach 10-15 cm (about 24-30 months on average) they are entering the male phase of sexual maturity and have attained relative immunity from common fish and crustacean predators. The clams may now be removed from the nursery cages and released unprotected on sandy or coral rubble substrates with a high probability of future survival (Heslinga et al. 1984). This is not to say that they may be completely ignored, however, as some episodic molluscan predators are capable of killing clams larger than 15 cm. At the MMDC clam nursery these predators are sufficiently rare that manual removal is an effective control measure.

The large muricid snail *Chicoreus ramosus* kills tridacnids up to 30-cm long by inserting its proboscis into the byssal opening (Heslinga et al. 1984). Because *C. ramosus* is conspicuous and slow moving, it is easily spotted during routine checks and can be removed from the nursery before doing much damage.

Large octupuses appear infrequently in the MMDC nursery, but are quite capable of killing clams in the 15-20 cm range. These predators attack by pulling the valves directly apart, leaving the hinge dislocated and the ligament partially or completely torn. A keen observer can sometimes follow a trail of recently discarded shells directly to the offending octopus, which should be speared. Large triggerfish which are also potential pests are likewise controlled by spearing.

The MMDC Five-Year Production Plan

An examination of current scientific and popular literature on tridacnid mariculture reveals widely disparate estimates regarding the production potential of hatcheries and ocean-based farms. While all of these projections are well-intended and arguably plausible, some are undoubtedly overoptimistic. Common sense suggests that conservatism should be the rule at this early stage. Moreover, it would be a service to those involved in research and development if the leading hatcheries provide timely, publicly accessible production reports.

The MMDC giant clam mariculture facility presently has 3 full-time staff, 6 larval tanks of 12-t capacity and 2,000 culture cages on a subtidal nursery of about 1 ha. Our near-term hatchery production quota is 100,000 *Tridacna derasa* seed per year, of mean size 10 mm at five months of age. The slower growing 50% of our annual seed production is discarded. Juveniles in the ocean nursery are transplanted and "split" to half their previous density every 5-6 months, and finally outplanted on the ocean floor at the age of 24-30 months depending on size. Based on available data, of the 50,000 juveniles planted annually, about 20,000 (40%) can be expected to survive to maturity and harvest at age six years postfertilization. The landed (in-shell) weight of 20,000 six-year old *T.derasa* is roughly 100 t, of which 75 t is dry shell, 1.6 t is adductor muscle and 10.7 t is other meat (Heslinga and Watson 1985). Based on present clam inventory (about 30 t not counting specimens previously sold or given to other groups) and assuming no change in size of hatchery or staff, we expect to harvest 10 t of clams in 1989; 50 t in 1990; 100 t in 1991 and 100 t each year thereafter. It can be readily seen that while we expect to produce considerable quantities of shell, our impact on the world market for tridacnid adductor muscle, at least in the next five years, will be minimal. Nevertheless, a production goal of 100 t/year is biologically challenging, commercially meaningful and logistically feasible for a small facility like the MMDC. The technology developed and experience gained from managing a program of this scale ought to be of regional interest and value.

In addition to producing commercial quantities of giant clam meat and shell, MMDC remains committed to its original objectives of using low technology methods wherever possible and making mariculture technology and training freely accessible to government and industry representatives throughout the Pacific Basin.

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Table 1. Cost summary of materials and labor required for the production of one giant clam nursery cage, based on lots of 1,000. (CIF Koror, Palau; in US$; 1984).

Component	Cost	
Tray		
Fiberglass mat (1 m^2)	$ 1.50	
Polyester resin (1 liter)	2.25	
MEK peroxide catalyst (4 cc)	0.04	
Mold and dowels	0.21	
Mold liner	0.09	
Aluminum legs	0.02	
Miscellaneous (brush, gloves, wax, (acetone, tools, etc.)	0.35	
Basalt gravel chips	0.17	
		$ 4.63
Lid		
Polyethylene mesh (1 m^2)	4.60	
Monofilament line; brass wire	0.10	
		4.70
Waste factor (5% of materials cost)		0.47
Labor		1.00
Total (materials plus labor)		$ 10.80

Table 2. Culture phases and suggested management protocol for annual production of 100 t of *Tridacna derasa*.

Culture phase	Clam age (months)	Size at start of phase (cm)	No. at start of phase		Density (No. per tray)	Projected selection to next phase
Larval	0-6	0.01	>10,000,000		–	<1.0%
Juvenile	6-8	1.0	100,000		200	50%
Nursery 1	8-13	2-3	50,000	(P)	100	75%
Nursery 2	13-18	5-6	37,500	(T)	50	75%
Nursery 3	18-24 (+)	7-8	28,125	(T)	25	75%
Grow-out	24 (+)–72	10-12	21,093	(O)	–	95%
Harvest	72	27.5	20,038		–	–

P – Plant; T – Transplant; O – Outplant.

Settlement and Growth of Oyster Spat in Trincomalee Bay, Sri Lanka

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Colombo 15
Sri Lanka

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Abstract

Oyster spat were collected from deep waters using floating rafts while stakes, racks and bamboo splits were used in the intertidal waters. Strings of asbestos plates were used as standard collectors with different space intervals for different sites. All organisms that settled on each collector were counted and a new string was introduced fortnightly. Growth of *Crassostrea madrasensis* and *Crassostrea belcheri* on plates and necessary hydrobiological parameters were monitored. Oysters spawned throughout the year with two peaks in November-January and June-August. The maximum mean number of spat on plates was 17.23/cm^2 and 5/cm^2 on stakes. A considerable amount of spat could be collected throughout the year from some locations. More spat settled on the under rather than the upper side of the plates. There were considerably more spat on the rough surface of bamboo splits than on the smooth surface, and the maximum collection could be obtained from submerged splits. Settlement of barnacles was considerable in some locations. *C. madrasensis* attained 80 mm after six months.

Introduction

Bivalve resources are considerable in the coastal waters of Sri Lanka, and many fishing families involved in deep-sea fishery turn to bivalve fishery when the sea is rough during the monsoon period. These protein-rich organisms are salted, dried and occasionally sold in some areas where they are plenty. An export market system for some bivalves in some areas of the country has just started. However, bivalve culture is still at the experimental stage in Sri Lanka where aquaculture plays an important role in fish production. Experiments on oyster, *Crassostrea madrasensis*, culture have proved the possibility of culturing these organisms successfully in waters with salinities of 10 to 50 ppt (Indrasena 1985). With the introduction of its culture in 1982, this oyster has become popular among people around culture areas.

Setting behavior, mode and time of settlement, as well as types of economically feasible collectors should be considered in commercial-scale oyster culture in addition to studying growth. This paper contributes some information on the availability and setting behavior of oysters in Trincomalee Bay with records on the growth of *C. madrasensis* and *Crassostrea belcheri* among eight other edible oyster types which are being identified.

Materials and Methods

Oyster spat were collected from nine sites in Trincomalee Bay, northeastern Sri Lanka, using floating rafts (4 x 4 m) in deep waters and racks, stakes and sticks in intertidal areas (Fig. 1).

Strings of asbestos plates (10 x 10 cm) were used as standard spat collectors and the plates were arranged with 1-m space between plates. For the collectors used in intertidal waters the space interval was 20 cm. During the setting season, the number of plates in each string of rafts was increased to 10 at 20-cm intervals; similar size rubber plates were used in the Clappenburg Bay for comparison. The total number of oyster spat settled on each plate was counted after two weeks. The collectors were replaced by a new string leaving the old string for spat to grow. The procedure continued from February 1984 to August 1985.

Asbestos plates were used in racks and bamboo splits (60 x 4 cm) were driven into mud as sticks to collect oyster spat from the intertidal region of the bay. Bamboo splits were arranged as three groups to cover the intertidal zone of the Mangrove Island, Snug Cove and Railway Cove (Fig. 1).

Just after the settlement of oyster spat two strings were selected from each site for growth studies. The number of spat on each side of the plate was limited to 5-10 by thinning. Total length and width of each oyster were then measured once each month and the plates were cleaned fortnightly to prevent interference in the growth of oysters by fouling organisms and new oyster spat. Specific growth of *Crassostrea madrasensis* and *Crassostrea belcheri* was calculated using the following formula:

$$G = \frac{(\log_e L_2 - \log_e L_1)}{(T_2 - T_1)} \times 100 \qquad \text{(Chatterji 1984)}$$

where L_1, L_2 are lengths (cm) and T_1, T_2 are time at the beginning and end of the experiment, respectively.

Results

Figs. 2-5 show the seasonal fluctuation of the amount of oyster spat settled in six different localities of the deep waters and in three different intertidal areas of the Trincomalee Bay.

Salinity, temperature and turbidity were measured and plankton samples were collected using a net with the mesh size of 55μm. As it was difficult to identify larvae of different oyster species and other bivalves, they were recorded as total bivalves per liter.

There was a significant relationship between the depth and number of spat at 5% level of significance. The spat of *C. madrasensis* and *C. belcheri* settled on surface layers in high densities. Almost all oyster spat that settled below 3 m depth were *Lopha* sp., *Plicatula* sp. and *Saccostrea* sp. A considerable amount of oyster spat was found on rubber plates during the peak season but this collection was very low compared to the settlement on asbestos plates.

At three sites (Mangrove Island, Railway Cove and Snug Cove) there was moderate settlement of spat on 60-cm bamboo splits. Settlement on the rough side (mean no. spat = 15.9/split) was significantly higher at the 5% level than on the smooth side (mean no. spat = 3.5/split). The sticks placed near the high tide mark showed little settlement. Those placed with their tops 20-cm above the low tide mark received most spat and the lowest 20 cm of the splits always received significantly more spat than the highest 20 cm.

Fig. 6a shows the growth increment and growth of *C. belcheri* grown on asbestos plates in Clappenburg Bay. Fig. 6b shows the same for *C. madrasensis* grown on plates in the Mangrove Island. *C. belcheri* grown on plates in Clappenburg Bay with the density of 8-10 oysters per plate showed an average length of 60 mm in one year. *C. madrasensis* grown on plates in Cod Bay with approximately the same density gave an average length of 70 mm. However, some individuals of *C. belcheri* reached 90 mm in one year; *C. madrasensis* reached 88 mm.

Fig. 6 shows the gradual decline in growth of *C. madrasensis* and *C. belcheri* with time and mortality. They grow rapidly with an average increase of 1.0 cm/month during first two months followed by a gradual decline.

Discussion

Oyster spat spawned throughout the year with at least two peaks in June-August and October-January. Comparatively poor collection in some sites may be due to the speed and direction of current, as at Nadithivu Island, or turbidity, as at Yard Cove.

Nicholson Cove recorded the highest collection (1,920 per plate) throughout the year. An extrapolated spat yield of 15.4 million could be expected from this site during the peak by the raft of 4 x 4 m with 20 plates in a string of 10-cm space interval and 40 cm between strings. The highest collection of *C. madrasensis* was from Mangrove Island and Clappenburg Bay. *C. madrasensis* and *C. belcheri* settled only in the peak season whereas *Saccostrea* sp., *Lopha* sp. and *Plicatula* sp. settled throughout the year at varying densities.

Significantly low collection of oyster spat on rubber plates compared to asbestos plates and bamboo splits indicate preference for rough and hard surfaces. Significantly higher collection of spat on the under side of the collector may be due to light avoidance and silt on the upper surface.

Salinity also played a vital role in the spawning of oysters, whereas temperature and turbidity were not significant.

Temperature generally remains above the critical levels in tropical waters and spawning is not directly related to temperature variations. Rapid changes in salinity are known to stimulate spawning activity of tropical marine invertebrates (Hornell 1910; Panikkar and Aiyar 1939). Rao (1951) suggested that the optimum salinity for spawning in *C. madrasensis* was 22 ppt; Joseph and Madhyastha (1984) observed 100% spawning at 32 ppt. In this study the peak settlement occurred one month after high salinity of 35-38 ppt. Salinity drifts, temperature and pH variations play a regulatory role in spawning periodicities, both directly and through the influence they exert on the biological community (Joseph and Madhyastha 1984).

Some individuals of *C. madrasensis* and *C. belcheri* on plates attained a length of 88 mm and 90 mm, respectively, at low densities. The growth rate of both species declined rapidly after nine months and was almost static or very slow after that. However, further studies on the density effect of oysters is necessary in order to determine the optimum number of spat to be left on plates for a commercial oyster culture.

Acknowledgements

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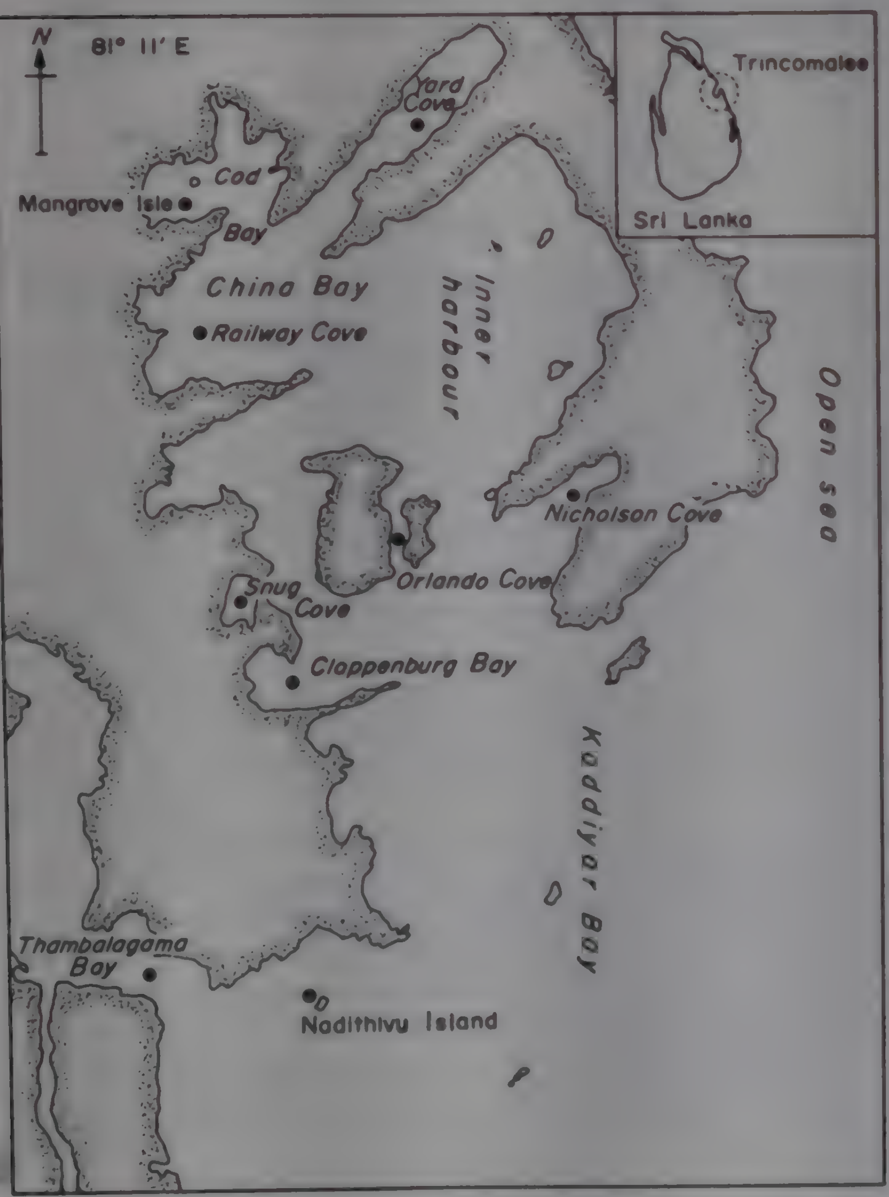
N
81° 11' E
Yard Cove
Cod Bay
Mangrove Isle
China Bay
Railway Cove
Inner harbour
Trincomalee
Sri Lanka
Open sea
Nicholson Cove
Orlando Cove
Snug Cove
Clappenburg Bay
Koddiyar Bay
Thambalagama Bay
Nadithivu Island

Fig. 1. Experimental culturing sites of the Trincomalee Bay.

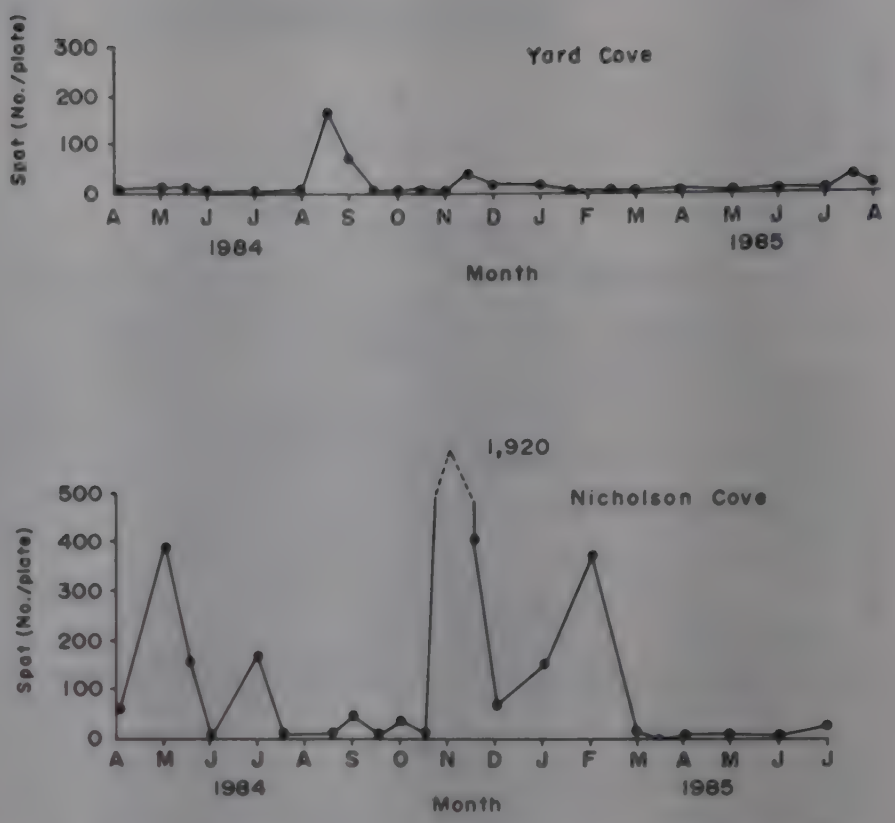

Fig. 2. Seasonal settlement of oyster spat at Yard Cove and Nicholson Cove sites.

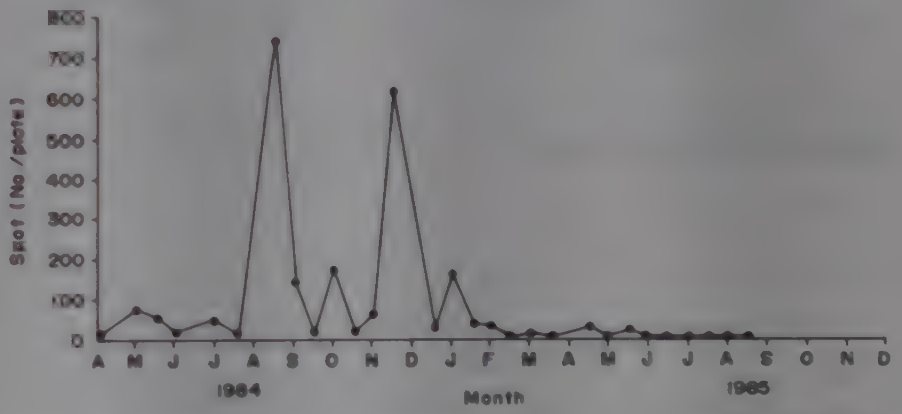

Fig. 3. Seasonal settlement of oyster spat at Clappenburg Bay.

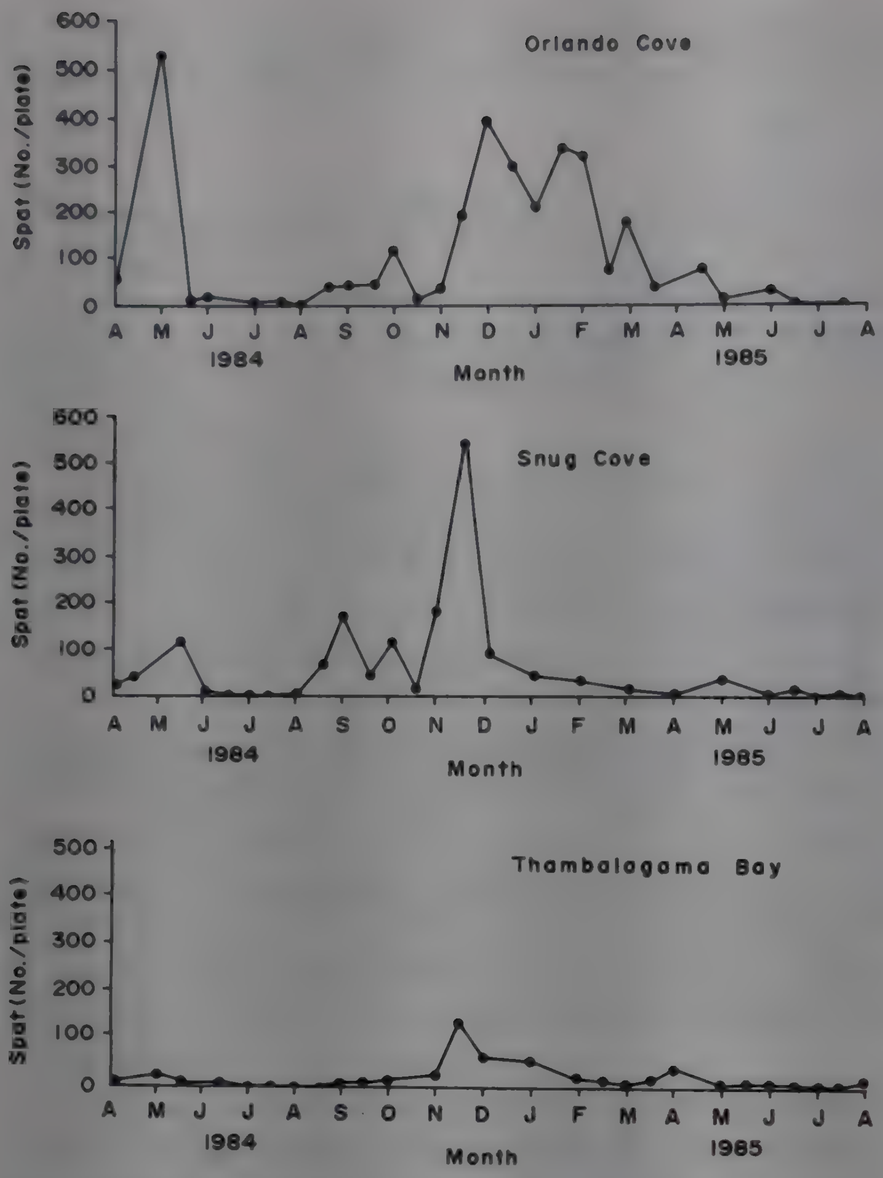

Fig. 4. Seasonal settment of oyster spat in Orlando Cove, Snug Cove and Thambalagama Bay.

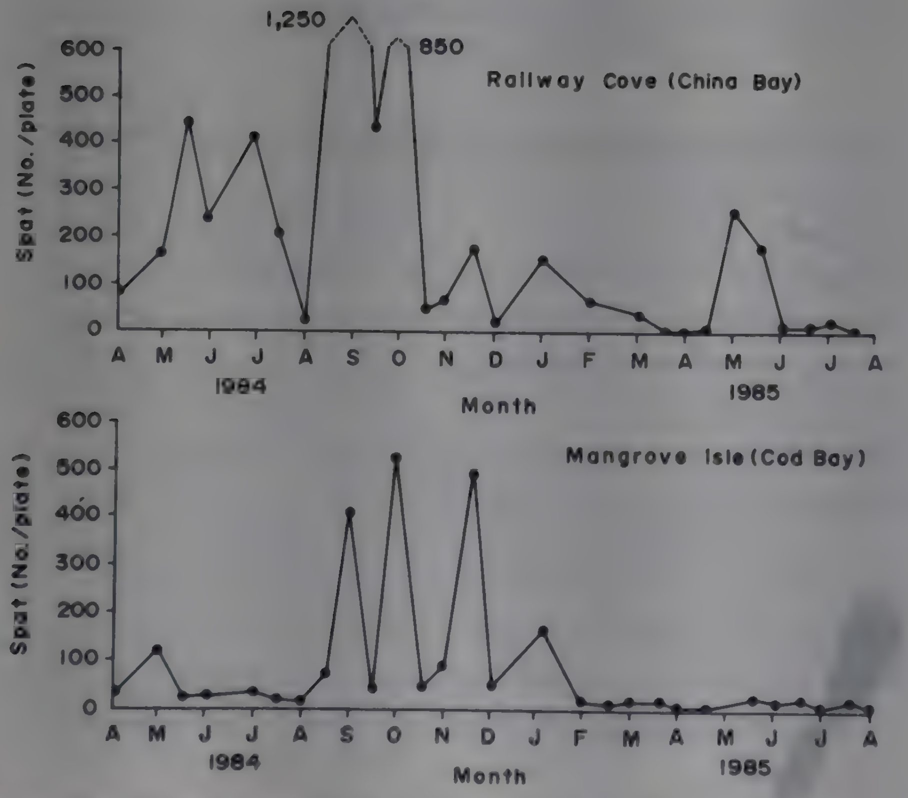

Fig. 5. Seasonal settlement of oyster spat in Railway Cove and Mangrove Island.

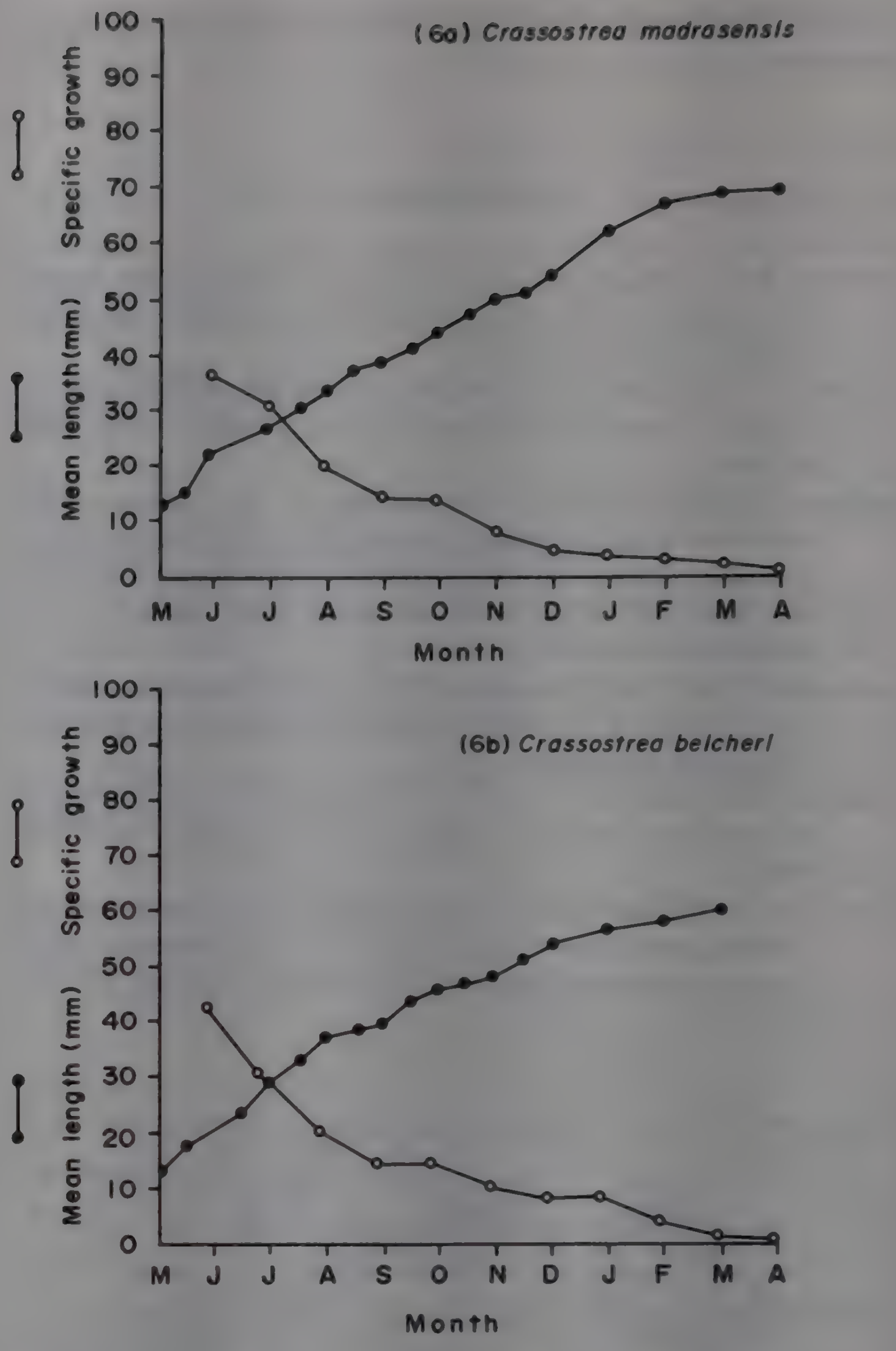

Fig. 6. Growth in length and specific growth of *C. madrasensis* and *C. belcheri*.

The Growth Rate of Milkfish, *Chanos chanos* in Brackishwater Ponds in the Philippines

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Abstract

Experimental data on mean weights of milkfish *(Chanos chanos)* taken at intervals during its culture from fry to fingerlings and from fingerlings to marketable juveniles in several yield experiments using organic and inorganic fertilizers at the Brackishwater Aquaculture Center, College of Fisheries, University of the Philippines of the Visayas in Leganes, Iloilo, reveal that absolute growth rate, a regression of mean weights to time, varies with pond carrying capacity and fish population density. Pond carrying capacity in this study was influenced by the type and quantity of natural food organisms (plankton and *lablab* or benthic organisms) in the ponds.

In nursery ponds fry were grown to fingerlings with an absolute growth curve that was exponential at stock density of 16,000/ha with *lablab* as food. At a higher stock density of 29,000/ha, fry growth was linear and slower.

Absolute growth of milkfish in rearing ponds from fingerlings to juveniles varied from power function, linear to log regression curve, depending on natural food biomass in the pond and fish stock density. At higher stock densities of 3,000-5,000/m^2, the absolute growth curve could be linear or log regression, indicating slower growth compared to steeper linear growth at lower stock density of 2,000/m^2. Better growth of milkfish was produced by *lablab* as food compared to plankton as evidenced by slightly steeper regression slopes of the growth curve in *lablab* ponds.

Introduction

Fish growth rate has been shown by many to be a logistic curve or sigmoid curve for both the individual organism or the population (Weatherly 1972). While the growth curve in natural populations follows the sigmoidal curve due to indeterminate growth of fishes, in fishponds where the food biomass and stock density are controlled by fish farmers growth shows a different pattern.

Schuster (1952, 1960), Rabanal et al. (1953), Juliano et al. (1970) and Grover and Juliano (1976) made preliminary studies on the growth rate of milkfish *(Chanos chanos)* in freshwater and brackishwater ponds, including its length-weight relationship. Grover and Juliano (1976) indicated that based on this relationship, fish are more robust when the regression coefficient is higher than 3.0 with *lablab* as food. Fish are heavier in the warm months of March to August than in the colder months of September to February. Juliano et al. (1970) showed a more or less linear growth rate of milkfish fingerlings to juveniles in freshwater ponds which was slower than in commercial brackishwater ponds. Schuster (1952) indicated that if stocking rate of fry is reduced to 1/m^2, slow, then accelerated, absolute growth to 30 g can be attained. Control of absolute growth rate can be practiced by fish farmers, either to accelerate or decelerate, by manipulating stock density or the quantity and quality of fish food (Juliano 1985).

This study was made to determine further the absolute growth curve pattern of milkfish in brackishwater ponds in relation to stock density, type of food (plankton and *lablab* or benthic organisms) and culture period in relation to temperature. The experimental data from yield experiments of the Brackishwater Aquaculture Center, College of Fisheries, University of the Philippines in the Visayas in Leganes, Iloilo, using combined organic and inorganic fertilizers to grow fry to fingerlings, and fingerlings to market-size juveniles are used in this paper.

Materials and Methods

The growth data available at the Brackishwater Aquaculture Center (BAC) were mean weights from different experiments, sorted and segregated in accordance with ecological conditions, fish food biomass growth, stock densities and fertilizers used. All absolute growth curve computations and plots as mean weights in grams in relation to time in days (corrected age in days) were done by Apple IIe microcomputers. There were no significant differences in fertilizer (chicken manure and commercial 16-20-0 and 18-46-0) dosage and type. The experiments were all conducted during the colder months of the year except for one experiment that ran through the warm months of March to May.

Five regression analyses, sigmoidal, linear, exponential, power curve and log regression on mean weight increments in relation to time with the best fit and statistical estimate, are used in this paper.

Results and Discussion

Growth rates of fry to fingerlings in plankton pond and *lablab* (benthic organisms) ponds at two stocking densities of 16,000 and 29,000/ha based on data with the best fit are presented in Table 1. With *lablab* as natural food at a stocking rate of 16,000/ha (1.6/m^2), the fry to fingerling growth curve was exponential. The confidence limits of the growth curves are acceptable for the three replicates. In 23 culture days, the fry grew to 7.1 gm. With such low stocking density, the exponential growth curve was expected. At a higher stocking density of 29,000/ha (2.9/m^2), with *lablab* also as food, growth was linear and slower. The third replicate, however, showed an exponential curve which means that the pond had probably more food available than the other two replicates.

Only one pond with plankton as food for fry to fingerling had data on absolute growth rate and the growth curve was a power function (an exponential type of growth). However, the linear regression line fits just as well as the power function with the same confidence limit.

With *lablab* as food, the pond with the lower stocking density of 1.6/m^2, had fry with exponential growth, growing to 7.1 g in 33 days, while in the pond stocked at 2.9/m^2, fry had linear growth to only 1.7 g in about 42 days.

At a stocking density of 2.9/m^2, milkfish fry seemed to grow slightly better with *lablab* as food rather than plankton. Statistically, however, the difference in the slopes of linear regressions (Table 1) was not significant.

The absolute growth rate data as analyzed by regression analyses showed variations even within treatments and between treatments (Table 2). These variations reflect certain factors influencing growth rates in milkfish from fingerlings to juveniles. It appears that, basically, stocking density and type of natural food have direct effects on milkfish growth. The milkfish growth curves in the newly-constructed Brackishwater Aquaculture Center (BAC) ponds are probably not as good as in many commercial fish farms as they sit on acid sulfate soil and in spite of liming before the experiments the effects of the fertilizers on primary production (whether *lablab* or plankton) were minimal. However, the trends indicated by the results of the regression analyses show certain significant aspects.

At stocking densities of 2,000 and 3,000/ha in *lablab* ponds, the fingerling growth rate was either linear or a power function (Table 2). At the higher stocking density of 4,800/ha, the growth curve was a log regression, meaning the growth at the start was fast and slowed down. The lowest stock density of 2,000/ha resulted in the biggest fish at the end of 132 days but the 3,000/ha density resulted in smaller fish than those in the 4,800/ha.

The growth data of milkfish from fingerlings to juveniles in deepwater plankton ponds were not consistent. At the lowest stocking rate of 3,000/ha, the growth curves varied from linear, log regression to power function. This indicates that the conditions in the ponds were not quite similar even between replicates within the same treatment. The predominance of the log regression growth curves in the series of deepwater plankton ponds at 3,000/ha indicates that the fish had reached the carrying capacity at the early stage of the culture period. It also means that the productivity of the ponds was quite low due to acidic soil.

At higher stocking densities of 5,000/ha and 8,125/ha which were carried out in privately-owned commercial ponds, the growth curves were power functions and linear, respectively. The ponds had high productivity to support a denser population than the BAC ponds. However, at a stocking rate of 7,800/ha in one commercial pond, the growth curve was log regression and the fish growth was depressed at a certain point. The variable and erratic results of the growth rates show that fish farms have different production capacities, even if located in the same place.

Table 2 shows growth in shallow-water plankton ponds of fingerlings to market-sized juveniles. At 5,000 to 8,000/ha, the fish growth showed a log regression curve, indicating regressive or retarded growth of the fingerlings during culture due to crowding. At one pond where only a 52.9% survival was obtained, a power function growth curve resulted, with a b value (slope) similar to those of the same stocking rate in *lablab* and deepwater ponds. At stock densities, however, of 5,000 to 8,000/ha, the b value of the growth curve (log regression) was much smaller than in the *lablab* and deepwater plankton ponds.

It seems that the carrying capacity of deepwater plankton ponds and *lablab* ponds are quite similar. However, the carrying capacity of shallow-water plankton ponds may be lower. The ideal stocking density in the latter may be 3,000/ha where a linear or exponential growth of milkfish fingerlings may be obtained. While this is probably true of the acidic soil condition of the new BAC fishpond system, much older ponds of commercial fish farms, where soil acidity has been rinsed away, may have a higher carrying capacity making possible higher stocking densities to attain desirable growth curves.

Only one experiment was in a cold culture period, from November to May. The regression analysis showed that the growth curve is a power function, slow at the start and increasing exponentially later. There is indication that lower temperature in "winter", (about 25°C) may have influenced fish growth because of lower metabolism and natural food production in the pond. Plankton production could have been lowered also by shorter daylight during the cold months because less solar radiation could enhance photosynthesis of the plant components of the plankton population. Villaluz and Unggui (1983) reported that milkfish fry had the lowest growth at a mean of 20°C while fastest growth was at 29.5°C.

In the experiment described above, the fish reached an average size of 370 g in 190 days. In the first 100 days of culture through the colder months, the fish grew from 5.86 g to about 110 g but in the next 90 days of warmer months, there was a net gain of about 260 g.

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Table 1. Absolute growth curves of milkfish fry grown to fingerlings in brackishwater ponds.

Stocking density	Growth	R^2	F	b	a	Culture days	Max. mn. wt.
Plankton pond							
29,000/ha	power	0.947	90.2	1.540	0.004	46	1.53
Lablab ponds							
16,000/ha	exponential	0.966	112.0	0.253	0.003	33	7.1
16,000/ha	exponential	0.981	260.1	0.246	0.003	33	7.1
16,000/ha	exponential	0.972	140.0	0.253	0.003	33	7.1
29,000/ha	linear	0.923	48.0	0.055	−0.273	38	1.83
29,000/ha	linear	0.935	71.7	0.044	−0.182	46	1.69
29,000/ha	exponential	0.961	99.0	0.191	0.002	38	2.45

Table 2. Absolute growth curves of milkfish fingerlings grown to juvenile in brackishwater ponds.

Stocking density	Growth	R^2	F	b	a	Days	Max. mn. wt.
Lablab ponds							
2,000/ha	linear	0.987	446.8	2.523	–129.539	132	207.1
2,000/ha	linear	0.950	115.2	2.476	–130.119	132	181.8
3,000/ha	linear	0.973	143.6	0.948	–47.122	153	90.6
3,000/ha	power	0.889	255.6	2.777	0.0	215	118.67
4,800/ha	log reg.	0.917	33.0	151.094	–590.669	136	130.4
4,800/ha	log reg.	0.922	35.7	142.452	–555.941	136	129.4
Deepwater plankton ponds							
3,000/ha	power	0.981	258.8	1.979	0.003	193	94.6
3,000/ha	linear	0.976	200.6	1.112	–54.441	193	147.9
3,000/ha	linear	0.970	159.9	1.196	–62.267	193	151.6
3,000/ha	log reg.	0.982	167.3	72.162	–280.712	123	65.8
3,000/ha	log reg.	0.986	214.3	76.938	296.375	123	73.0
3,000/ha	linear	0.989	266.7	1.687	–87.437	123	120.0
3,000/ha	log reg.	0.969	62.8	208.732	–843.471	152	190.7
3,000/ha	log reg.	0.997	573.7	182.214	–741.256	152	178.4
3,000/ha	log reg.	0.945	187.4	86.188	–330.188	212	125.67
*5,000/ha	power	0.993	999.9	2.712	0.0	242	370.0
7,800/ha	log reg.	0.922	58.8	153.967	–618.513	221	242.0
8,125/ha	linear	0.991	458.2	1.227	–58.387	160	133.0
Shallow-water plankton ponds							
5,000/ha	log reg.	0.883	15.0	57.716	–209.982	139	67.5
5,000/ha	linear	0.967	59.5	0.976	–35.236	139	101.2
5,270/ha (90%)	log reg.	0.972	246.7	81.942	–319.757	171	100.0
5,270/ha (52.9%)	power	0.937	104.5	181.64	–737.602	171	206
8,000/ha	log reg.	0.991	15.0	52.820	–192.221	139	67.5
8,000/ha	log reg.	0.919	229.8	37.369	–125.366	139	56.3

*Cultured November to May, passing through the cold months of December through February.

Mass Production of Seabass, *Lates calcarifer* (Bloch), by Environmental Manipulation

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Abstract

Seabass, *Lates calcarifer* (Bloch), is an important aquaculture species in many Southeast Asian countries. The fish has been successfully spawned by hormonal manipulation since 1973. To minimize the use of expensive hormones as well as stress on the fish through handling and hormonal injections, environmental manipulation, mainly through control of salinity and temperature to induce spawning in captivity, was used. With this method, some 50-100 million fry and fingerlings were produced per year continuously at the Satul Fisheries Station in Satul, Thailand. This report describes the methodology of environmental manipulation, prespawning behavior, larval rearing techniques and monthly production of seabass.

Introduction

Seabass, *Lates calcarifer* (Bloch), has been commercially cultivated in brackishwater ponds and marine cages in Thailand, Malaysia, Taiwan, Singapore, Indonesia and Hongkong. As seabass is carnivorous, its cultivation is dependent upon adequate supply of trashfish and compound feeds. The seabass is a relatively highly priced and widely accepted species and therefore has become a very attractive commodity for both large- and small-scale fish culture enterprises.

The seed used for culture are usually obtained from the wild. The availability of fry from natural collecting grounds, however, fluctuates widely from year to year, making its supply very erratic and inconsistent. To ensure regular and adequate supply of seed for culture activities, efforts have been made by many researchers in Southeast Asian countries to reproduce them under controlled conditions. Artificial propagation of seabass was first achieved in Thailand in 1971 by stripping the ripe spawners collected from natural spawning grounds. In 1973, Wongsomnuk and Maneewong (1974) successfully induced cultured broodstock to spawn in captivity by hormone stimulation. However, with this method, handling causes stress in the fish, resulting in broodstock mortality.

Years of experience in seabass fishing have taught the Thai fishermen that the spawning season of seabass on the west cost of Thailand facing the Indian Ocean occurs during the southwest monsoon season (April-August) when there is slight rainfall. This is confirmed by the availability of 1-cm long fry in collecting grounds from May to August (Bhatia and Kungvankij 1971). Prior to the spawning season, the spawners migrate to the mouth of rivers or lakes where the salinity is 30-32 ppt. The fish spawn between the onset of the new or full moon and for the succeeding seven days. Spawning occurs during the late evening (1800-2200 hr) at incoming tide (Kungvankij 1981).

This paper reports the experiments conducted at Satul Fisheries Station, Satul, Thailand, on the induction of seabass spawning by environmental manipulation to minimize the use of expensive hormones and stress on the fish through handling and hormone injection. The experiment used the above biological and ecological information obtained through field observations and data on the natural occurrences of fry. Environmental manipulation to stimulate the fish to spawn in captivity included: (1) changing the water salinity to simulate the condition for fish migration from lower to higher salinity; (2) decreasing the water temperature to simulate the drop in water temperature due to rainfall; (3) adding fresh seawater to the holding tank to simulate the rising tide; and (4) conducting these manipulations close to the new or full moon periods.

This paper also presents the results of studies on the larval rearing and the mass production of fry and fingerlings of *Lates calcarifer*.

Materials and Methods

Juvenile seabass were reared in cages to adult size (3-yr old, 4-5 kg). The history and physical condition of the broodfish were monitored.

Broodfish were reared in floating cages (5 x 5 x 2 m) anchored at La ngu Bay, opposite Satul Fisheries Station. The cages were made of polyethelene netting attached to galvanized iron pipe frames kept afloat by styrofoam drums. The mesh size of the net used varied depending on the size of fish: 1-2 cm mesh for juvenile and 4-8 cm for older fish. Juvenile fish (5-10 cm) were stocked in the cage at 50 fish/m^3. The fish were graded monthly to select healthy and fast-growing fish for broodfish. One year-old fish (1.2 kg) and two year-old fish (2.5 kg) were stocked in these cages at 2 and 1/m^3, respectively. After a culture period of about three years, 48 spawners were selected from the broodstock cages. The average weight of the fish was 4 kg. Two months before the spawning season, the selected broodfish from the cages were transferred to the spawning tanks at 24 fish/tank. The sex ratio was 1:1. The spawning facilities consisted of two 100-t rectangular concrete tanks (5 x 10 x 2 m), equipped with water inlet and outlet and an aeration system. Some shading with roof tiles was provided to protect the fish against strong sunshine and heavy rains.

Immediately after stocking in the spawning tanks, the feeding rate was reduced from 5%/day to 1%/day total body weight. The feeds given were fresh marine fish such as *Clupea* spp., *Selaroides* spp. and *Nemipterus* spp.

The water quality in the spawning tanks was maintained by changing 50-60% of the tank water daily.

The initial salinity of the water in the spawning tanks was 20-25 ppt. One week after stocking, 50-60% of the water was changed daily with fresh seawater until the salinity reached 30 or 32 ppt to simulate the natural conditions the fish encounter during migration from nursery to spawning ground.

At the start of the new or full moon, the water in the spawning tanks was lowered to about 30 cm deep at noon and left exposed to the sun for two to three hours. The water temperature in the spawning tank would thus increase to 31-32^{o}C. New filtered seawater was rapidly added to the tank to simulate the condition of rising tide. This process drastically decreased the water temperature to 27-28^{o}C.

The fish spawned immediately the same night or the next after the environmental manipulation at 1800-2200 hr. If no spawning occurred, the above manipulations were repeated for two to three more days.

Fertilized eggs were collected from the spawning tanks with a fine mesh size dip net the morning after spawning. During egg collection, planktonic organisms adhering to the eggs were removed by filtering the eggs repeatedly through a series of screens. Unfertilized eggs which settled at the bottom of the hatching container even under mild aeration were removed by siphoning.

The eggs were then transferred either to hatching containers if the numbers were small or directly to nursery tanks if the number were large for mass production. The larvae were later transferred from the hatching containers to nursery tanks. Hatching containers consisted of cone-shaped fiberglass tanks with a capacity of about 1,000 l. Water in these tanks was aerated by a lead-weighted airstone.

Thirty outdoor rectangular concrete tanks (1.5 x 10 x 1 m) each with 15 m^3 were used for larval rearing. The tanks were protected from rain and strong sunshine by roof tiles.

The usual density for newly-hatched larvae in the rearing tank was about 100 fry at yolk stage per liter.

During the first three days after hatching, the larvae were not given any feed as they still fed on the yolk sac. However, single-celled algae *(Chlorella* spp. or *Tetraselmis* spp.) were added on the first day of rearing to maintain good water quality.

Three days after the yolk had been fully absorbed and the mouth was fully developed, rotifers *(Brachionus plicatilis)* were introduced as feed. A density of 5-10 rotifers/cm^3 was maintained for about a week.

The larval density was then reduced to about 40 larvae/l by transferring some of the larvae to another nursery tank. The diet of the larvae then changed to brine shrimp *(Artemia* sp.) nauplii for about 10 days. Thereafter, bigger-sized artemia were fed to the fry for another 10 to 20 days. When the fry attained 12-15 mm body length, (about 30 days old) they were given ground fish meat.

Seabass is a carnivorous fish and cannibalism is distinctly rampant from the time the larvae start to feed on artemia. Grading was done a week after the fish began to feed on artemia and every week thereafter.

Grading trays used in the experiment were plastic basins with many holes bored through the bottom of size 3 mm to 10 mm for specific size of fish to pass through.

The newly-hatched larvae were reared in filtered seawater with very mild aeration. Unfertilized eggs, feces and excess feed accumulating at the bottom of the tanks were siphoned out daily. At the stage when the fish were fed artemia, one third of rearing water was changed daily. When the larvae started eating minced fresh fish, running water was applied to avoid water quality problems.

Results

The fish reared in the net cages attained an average weight of 1.2 kg after one year, 2.5 kg after two years and 3.5 kg at maturity after three years.

The fish spawned immediately the night after environmental manipulation (1800-2200 hr). In some cases, environmenal manipulation was repeated for one or two more days. After the first spawning, spontaneous spawning continued for three to five days without further manipulation. Two to three days before the new or full moon, there was an increase in prespawning play activity. The ripe male and female swam together more frequently near the water surface as spawning time approached. Spawning of the same fish was repeated on the same day of the full or new moon (or days thereafter) over the following 5-6 months (Table 1). The ranges of salinity and temperature in relation to spawning and hatching success of seabass are shown in Fig.2.

Hatching rates obtained were 40%-85% (Table 1). The survival rate varied from stage to stage, about 35% from yolksac fry to fry, about 60% from fry to 1-cm fish and 45% from 1-2.5 cm fingerlings.

Table 1 shows that the first period where high mortality occurred was between days 3 and 5. The second period of high mortality was during early fingerling stage (days 25-30, 1 cm in length) when larval food was changed from live feed to trash fish. The overall survival rate from yolksac fry to 2.5 cm fingerlings was about 15 to 20%.

Seabass fry were separated into three categories according to the size requirements of the farmers (Table 2).

Out of 63 million yolksac fry produced by 24 pairs of spawners in two 100-m^3 spawning tanks, only 34 million of different larval stages were distributed to the farmers and fisheries stations and for open water stocking. Of these, about 24 million were yolksac fry, 9 million fry, 0.45 million 1-cm fingerlings and 0.6 million 2.5-cm fingerlings (Table 1).

Discussion

Ideally, spawners should be 4-5 kg in body weight and at least three years old. Males and females of about the same age group and size are preferred. About two months before spawning, broodfish reared in the cages are selected and transferred to spawning tanks. The ratio of male to female is 1:1. The spawners are selected according to the following criteria: (a) fish should be active; (b) fins and scales complete; (c) free from diseases and parasites; and (d) no injury or wounds.

Seabass is one of the marine fishes difficult to sex by external characteristics, except during the spawning season. However, there are some distinguishable characteristics: (a) snout of male fish can be slightly curved while that of the female is straight; (b) the male is more slender, so that body depth of male is less than that of female; (c) females are heavier than males of the same size; (d) the scales near the cloaca of the male are thicker than those of the female during the spawning season; and (e) the abdomen of females is bulging.

Constant monitoring of fish is required to detect prespawning activities. Two weeks to one month prior to spawning, the shoal of fish in the tank begin to swim to the surface and their silver belly can be seen as they begin their descent. They do so more often when they are ready to spawn.

About one week prior to spawning, the female fish separates from the shoal and ceases feeding. As the female approaches spawning time, there are increases in play activities.

Under confinement, competition among the individuals for feed and space occurs, resulting in uneven growth. If the stock is poorly managed, heavy mortalities will occur. This may be due to cannibalism or stress especially on the small or weaker fry. To avoid cannibalism, size grading should be done and graded fish reared separately.

The other factor which causes high mortality is disease. The most common symptoms of disease in seabass fry are: (a) loss of appetite; (b) change of body color from gray to black; (c) loss of scales; and (d) white spot formation.

Treatment should be done immediately after the appearance of these symptoms. Suitable treatments include immersion of the fry in water at reduced salinity of 15 to 20 ppt with 20 ppm formalin for one to two hours and immersion of fry in 3 ppm oxytetracycline for 10 hours.

Both treatments should be done continuously for three to five days until the larvae regain their normal coloration and appetite.

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Table 1. Hatching and survival rate in each stage.

Month	Eggs (x 10^3)	Yolksac fry (x 10^3)	Hatching rate (%)	Fry (x 10^3)	Survival rate of yolkfish to fry stage (%)	1-cm fingerling (x 10^3)	Survival rate of fry to 1-cm (%)	2.5-cm fingerling (x 10^3)	Survival rate from 1 to 2.5-cm fingerling (%)
April	5,200	4,200 (1,000)	80.7	2,100 (1,800)	65.6	250	83.3	151	65.3
May	6,120	4,710 (3,280)	76.9	650 (450)	45.5	126 (2)	63.0	41	33.0
June	7,860	6,150 (3,400)	78.2	1,350 (805)	49.1	376 (230)	68.9	11	7.5
July	12,590	10,000 (4,000)	79.4	2,340 (400)	39.0	940 (50)	48.5	100	11.2
August	16,050	12,650 (5,400)	78.8	2,880 (1,300)	39.7	820 (25)	51.9	50	6.3
September	2,110	(1,200)	50.9						
October	2,520	1,917 (1,000)	76.7	418 (400)	45.6	82	45.5	4	48.8
November	390	272	69.7	86	31.6	53	73.3	30	56.6
December	1,700	1,215	71.5	402	33.1	21	53.7	13	14.5
January	200	86	43.0		DISCARDED				
February	1,438	1,140	79.3	415 (200)	30.2	152	70.6	74	48.6
March	3,770	2,960	78.5	1,295 (1,158)	43.7	92	67.2	59	64.1
April	6,640	4,890 (2,500)	73.6	566 (430)	23.6	82	60.2	8	10.4
May	1,400	11,950 (2,000)	85.4	3,160 (2,040)	31.7	480 (4)	42.8	5	11.3

Notes: Figures in parentheses are fish distributed to institutions and private hatcheries. Survival rates are based on the number of fish reared in the hatcheries after disposal.

Table 2. Criteria for classification of seabass fry size group.

Grade	Age (days)	Length (mm)	Feed preference
Yolksac fry	1-2	1.8-2.5	*Brachionus*
Fry	10-15	3.5-5.4	Artemia
Fingerling	30-45	10-25	Adult Artemia trash fish

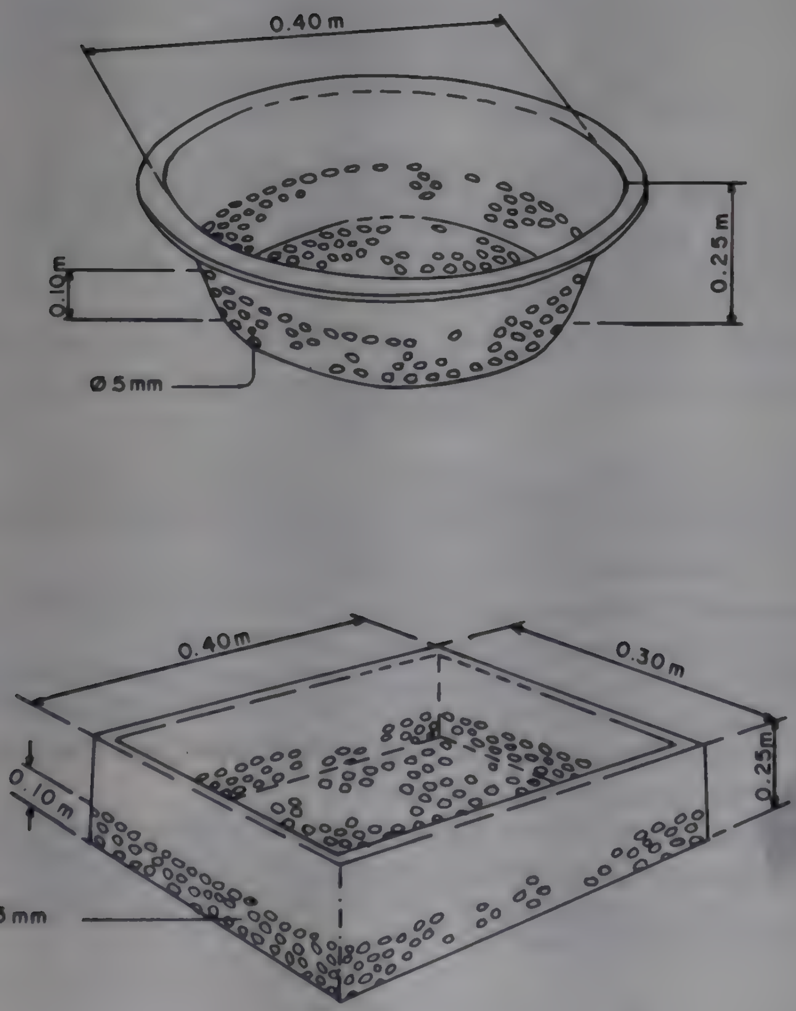

Fig. 1. Grading vessels for seabass fry.

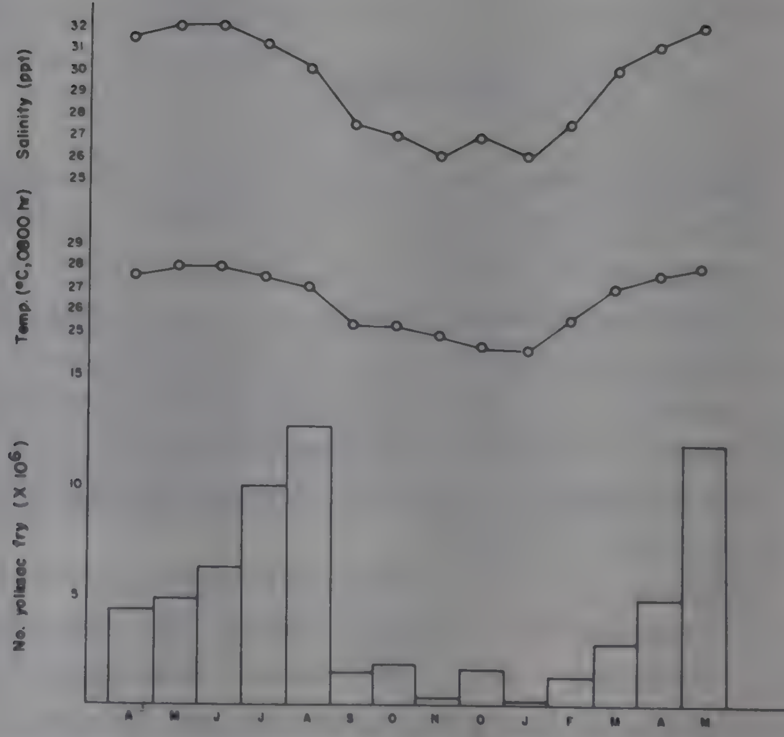

Fig. 2. Salinity and temperature in relation to spawning and hatching of seabass.

Acidification and Reclamation of Acid Sulfate Soil Fishponds in Thailand*

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Abstract

An experiment was conducted to examine the chemical characteristics of and the effects of fertilization on water quality and biological productivity in fishponds built in the acid sulfate soil region of Thailand. The acid soil acidified the overlying pond water rapidly to pH less than 4, but its acidification effect was reduced remarkably by repeated changing of the pond water with alkaline source water. Further improvement of the pond water was done by liming and enriching the ponds with inorganic and organic fertlizers. The pH in ponds receiving inorganic fertilizers ($N_{16}P_{20}K_0$) fluctuated widely necessitating repeated liming. Production of food organisms (phyto- and zooplankton) was relatively poor. Fish yield (*Oreochromis niloticus* and *Puntius gonionotus* stocked at a density of 3 fish/m^2) in five months was only 426 kg/ha. In comparison, the pH in ponds fertilized with chicken manure stabilized in the alkaline range and fluctuated little after initial liming. Relatively high plankton productions were achieved and fish yield was 1,528 kg/ha. Methods for reclaiming the acid soils for productive fishponds are recommended.

Introduction

Acid sulfate soils cause the acidification of large areas of surface water in the tropical ecosystems (Brinkman 1982). The worldwide extent of acid sulfate soils is estimated to be 13 x 10^6 ha of which 778,000 ha are located in Thailand's central plain (Pons 1969). Those soils contain large concentrations of sulfate and pyrites, creating extremely acid soils and surface waters in the region (Attanandana et al. 1982). In addition, the acid waters also contain free aluminum ions which are highly toxic to fish and form precipitates with phosphates making the fertilizer unavailable to organisms in the environment. Consequently, the surface waters affected by the acid soils are biologically unproductive. The application of lime and fertilizers greatly increased fish productivity in acid ponds in Malaysia (Hickling 1968).

Reclamation and utilization of acid sulfate soils for aquaculture are well documented for the coastal regions in the Philippines where the potential soil acidity could be leached and removed by tidal flush (Brinkman and Singh 1982; Singh 1985). However, in the flat terrain of freshwater regions, as in Thailand's central plain, such natural mechanism does not occur. To achieve this artificially is technically difficult and expensive, and the leached effluent may cause damage to adjacent surface waters.

This work reports the acidification of pond water caused by acid sulfate soils and the impact of inorganic fertilizers on plankton and fish production in those fishponds.

Materials and Methods

The experiment was conducted at the Nong-Sua Fisheries Station located in the lower part of Thailand's central plain where severe acid sulfate soils prevail. To examine the depth of some acid-related chemical features, soil samples taken in a 1-m core with a soil auger were sectioned in 20-cm intervals from surface to bottom. Each section was analyzed for pH, active iron, extractable aluminum, calcium, sulfur, phosphorus, and % base saturation. The effectiveness of leaching and washing processess for removing acidity from soil was determined in a small-scale experiment. Six enamel-coated containers (each 100 cm long, 40 cm wide and 50 cm deep) were filled with 20 cm of bottom soil taken from a new pond. Upon drying the soil, the containers were filled with alkaline water (pH 7.3) to 30 cm above the soil surface. The pH changes of the overlying water in the containers were recorded daily for four consecutive days, then water was drained and soil dried. Again, the tanks were filled with new source water and the pH was monitored for another five days.

A field experiment for determining the effect of inorganic and organic fertlizers was carried out in four 800-m^2 earthen ponds. Two received inorganic fertilizer ($N_{16}P_{20}K_0$) and the other two, organic fertilizer (chicken manure). The nutrient input from the inorganic and organic sources into each pond was calibrated at a rate of 8 kg P_2O_5/ha/month. Bottom soil analysis showed that the initial quantity of lime applied to the bottom ranged from 5,000 to 6,800 kg/ha.

Two species of herbivorous fish, *Oreochromis niloticus* and *Puntius gonionotus*, were stocked in the experimental ponds at 2/m^2 and 1/m^2, respectively. During the five-month grow-out period we collected weekly data on pH, alkalinity, dissolved oxygen and chlorophyll *a* in the ponds. Fish growth was determined by monthly samples of 10% of the population, and zooplankton standing crop was sampled monthly.

Results and Discussion

The depth profile of soil chemical characteristics showed decreased values of pH, calcium, base saturation and phosphorus as depth increased in the top 1-m soils. The opposite trend existed for iron, aluminum and sulfur with increased concentrations in deeper soil strata (Table 1). Those data indicate that the potential acidity in the soil increases with depth in the acid sulfate soil, suggesting severer acid problems would be encountered with deeper excavation in pond construction.

The results of the small-scale experiment on acidification showed that the pH of alkaline water decreased rapidly from 7.3 to 3.5 in 3-4 days, but the acid exported from the acid soil diminished greatly upon repeated water changes (Fig.1). The potential acid in the top soil could be reclaimed by repeated leaching and washing. However, mechanical pumping and draining of the pond is costly. Furthermore, the effluent from the leached acid water would cause damage to the organisms in the adjacent recipient waters.

The effects of inorganic and organic fertilizers on water quality and biological productivity in the acid soil ponds were remarkably different. The pH and alkalinity in the ponds receiving chicken manure were considerably higher (Fig. 2). Fluctuations in pH and alkalinity were particularly pronounced in the rainy season during which the pH in the inorganic fertilized ponds dropped below 6 and additional limings were required to maintain the pH desired for fish growth. The application of organic fertilizer in the acid sulfate ponds may involve significant biochemical processes that stabilize the pH in the pond bottom. The continuous deposition and decomposition of organic matter at the pond bottom create low redox or anaerobic conditions in the surficial sediment and its interstitial waters. These biochemical processes can prevent oxidation and lock the reduced sulfur compounds in the sediments. Furthermore, the low redox conditions would enhance the nutrient release and recycle, as well as reduce sulfate to highly insoluble ferrous sulfides from the sediments. The aluminum toxicity to fish, while most potent in its inorganic forms, may be averted as the aluminum ions are complexed by the large amount of soluble organic compounds (Driscoll et al. 1980). The drastic pH decrease in pond water during the rainy period was primarily caused by the runoffs from the pond dikes (Simpson et al. 1983; Singh 1985). Prevention of acid leaching from dikes is difficult because its periodical episodes effectively oxidize pyride; limings are ineffective as lime is easily washed off by rains and a new layer of soil is exposed.

As measured by chlorophyll *a*, phytoplankton production in the chicken-manured ponds ranged from 20 to 120 mg/m^3 compared to 5-30 mg/m^3 in the inorganically fertilized ponds (Fig. 3). Pronounced differences in zooplankton (mainly protozoans and rotifers) also occurred between ponds receiving the two types of fertilizers (Fig. 4).

Fertilizer effects on the fish production are presented in Table 2. Although the two species of fish were stocked at similar sizes (0.26-0.37 g/fish), the survival rates were significantly higher for *Puntius* (97-100 %) than those for *O. niloticus* (60-82 %) in both types of ponds. While the *Puntius* encountered little mortality, its final yields were only 92 and 234 kg/ha in inorganically fertilized and chicken-manured ponds, respectively; *Tilapia* yields were 334 and 1,294 kg/ha, respectively. In the Philippines, maximum milkfish yield in reclaimed acid ponds was 550 kg/ha in a three-month growing period (Singh 1985).

Management Implications and Recommendations

Depth profiles of acid related chemical features in the acid soil such as pH, aluminum, iron and sulfur indicate that digging should be avoided in pond construction. Instead dikes should be built on top of the original terrain with the top soil saved from digging the drainage canals. To reduce the amount of dike runoffs relative to the pond water volume, one should reduce the size of the dikes, increase the pond size and maintain high pond water level. The top surface of the dikes should be built in "V" shape with a central depression to collect excess rain water and empty it into the drainage canal. The ponds should be fertilized with animal manure to enhance greatly the production of food organisms, as well as to improve water quality by stabilizing the pH.

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Table 1. Depth profile of soil chemical characteristics at Nong-Sua Fisheries Station, Thailand central plain.

Parameter	Depth (cm) 0-20	20-40	40-60	60-80	80-100
pH	4.0	3.7	3.5	3.4	3.2
Active Fe (%)	0.7	0.7	3.0	3.4	2.9
Extractable Al (meq/100 g)	6.7	16.7	16.1	14.6	14.6
Calcium (%)	0.27	0.11	0.09	0.09	0.09
Sulfur (%)	0.49	0.17	0.43	0.66	0.78
Phosphorus (ppm)	310	155	130	110	105
Base saturation (%)	46	32	28	29	28

Table 2. Stocking density, survival rate, weight gain and total production of two fish species cultured together for 180 days in ponds treated with organic and inorganic fertilizers.

Fertilizer and fish spp.	Stocking rate (fish/m^2)	Survival rate (%)	Wt. gain (g/fish)	Yield (kg/ha)
Inorganic				
Puntius gonionotus	1	97	9.5	92
O. niloticus	2	60	27.4	334
Chicken manure				
Puntius gonionotus	1	100	23.4	234
O. niloticus	2	82	78.0	1,294

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*Contribution No. CRSP 86-8.

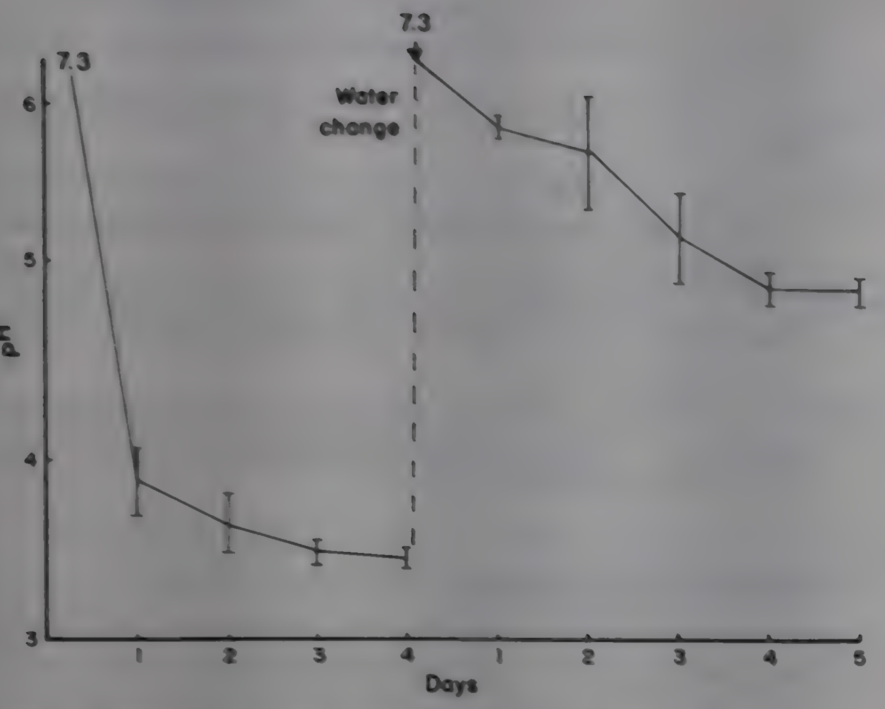

Fig. 1. Effects of acid sulfate soils on the alkaline pond water upon two successive water changes.

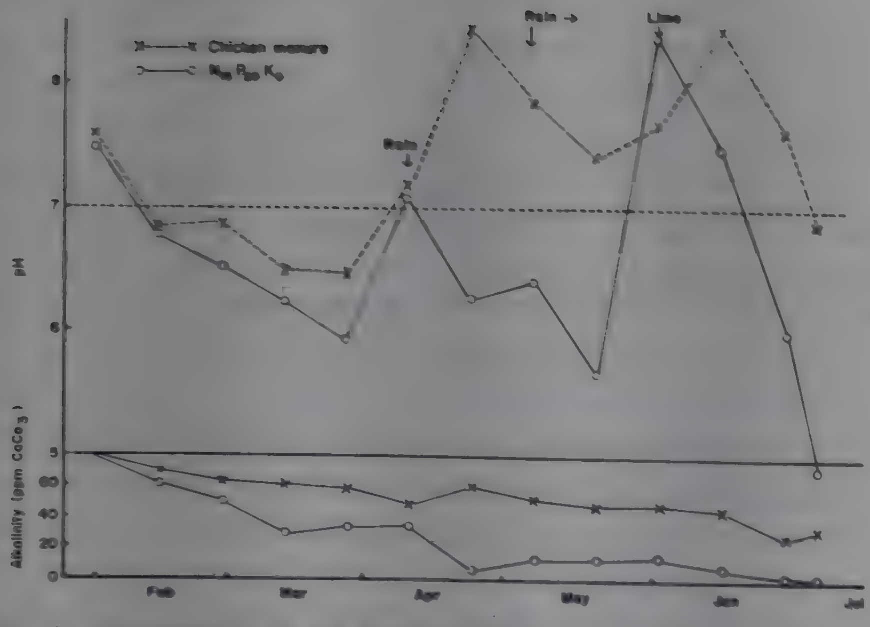

Fig. 2. Comparison of pH and alkalinity between inorganic and organic fertilized ponds.

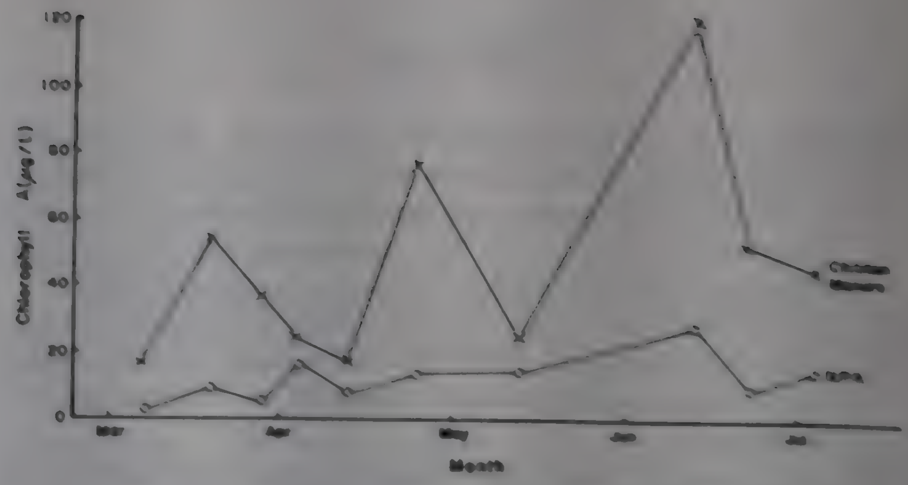

Fig. 3. Comparison of phytoplankton production (chlorophyll a) between inorganic and organic fertilized ponds.

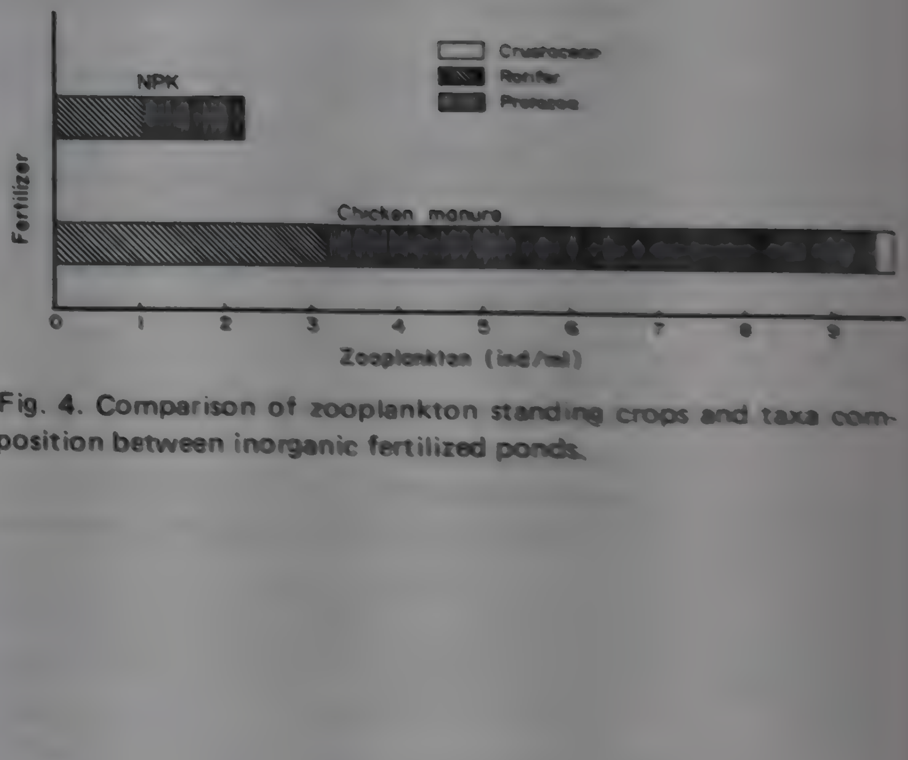

Fig. 4. Comparison of zooplankton standing crops and taxa composition between inorganic fertilized ponds.

The Philippine National *Bangus (Chanos chanos)* Breeding Program

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Abstract

The National Bangus Breeding Program (NBBP) was implemented by the Bureau of Fisheries and Aquatic Resources in 1981 in twelve stations throughout the Philippines. Milkfish or *bangus*, *Chanos chanos*, broodstock were one-year old juveniles either grown in ponds maintained by the project or purchased from cooperators. Some 250 juveniles were stocked in each of the 10-m diameter circular floating cages. The fish were fed commercial formulated diets (fish pellets) with 20% protein content at 1.5-2.0% of the body weight given twice daily until they were three years old. The broodstock were then given crustacean feed pellets with 42% protein level at 2-3% body weight also given twice daily. Representative sampled specimens were sacrificed once the broodstock reached their 4th year to monitor gonadal development and to determine gonadosomatic indices (GSI). Early maturing fish were obtained in the samples in March and August 1985. Initial fecundity counts showed a range of 660,000-1,100,000 eggs per female. These results were obtained in the third and fourth years at Regions I (Alaminos, Pangasinan), III (Masinloc, Zambales) and XI (Sta. Cruz, Davao del Sur) stations. Samples at the other stations indicated a preponderance of developing to early maturing gonads.

Introduction

Faced with the prospect of a limited fry supply, aquaculture researchers in the Philippines and in other countries have increased efforts at artificially propagating milkfish. These efforts resulted in successful induced spawning of wild adults (Kuo et al. 1979; Liao et al. 1979) and captive broodstock (Juario and Natividad 1980); Liao and Chen 1983) as well as development of larval rearing techniques that could yield survival rates of up to 70% (Vanstone et al. 1977; Juario et al. 1984). However, due to difficulties in obtaining wild spawners it became essential to establish and maintain captive broodstock. In the Philippines, milkfish breeding in captivity was pioneered by the Southeast Asian Fisheries Development Center, Aquaculture Department (SEAFDEC-AQD) and has resulted in the maturation, spawning and successive rematuration of second generation milkfish breeders (Anon. 1985).

In other Southeast Asian countries, milkfish breeders were successfully made to spawn spontaneously, both naturally and by stimulation under captive conditions using as holding facilities either earthen ponds as in Taiwan (Kuo 1984; Lin 1985) or in canvas and concrete tanks as in Indonesia (Poernomo et al. 1985). These developments have assured the potential for increasing milkfish fry supply through controlled breeding.

As an ongoing activity of the Philippine Bureau of Fisheries and Aquatic Resources (BFAR), the lead agency implementing this program, the National Bangus Breeding Program (NBBP), primarily draws on the milkfish breeding experience of SEAFDEC-AQD. Its aim is to verify on a national scale and in varied ecological conditions the successful research work of the latter on milkfish breeding conducted at its Igang Research Station.

Materials and Methods

Site survey was undertaken to identify sites suitable for milkfish maturation and breeding activities. The following criteria were considered: (a) protection from wind and wave action even under adverse weather; (b) good water circulation; (c) minimum water depth of 5 m at lowest low tide; (d) sandy-muddy substratum; (e) salinity range of 28-35 ppt; (f) water temperature range of 25-34°C; (g) minimum water transparency of 3 m; and (h) a considerable thriving milkfish fry industry which would directly benefit from this project.

The milkfish broodstock were one-year old juveniles grown in ponds maintained by the project or from six-month to one-year old juveniles purchased from cooperating fishpond operators.

The rearing ponds used in the project are similar in design and construction to ordinary brackishwater ponds used in milkfish culture. Each pond was 1 ha with a water depth of about 0.5 m and prepared following currently-employed procedures recommended by Lijauco et al. (1979). The stocking and rearing procedures varied according to region/station. Stations with existing ponds either stocked them with fry collected from the wild or grew the fry in a cooperator's fish farm to juvenile size prior to rearing in their own grow-out ponds for one year. The stocks were made to depend solely on natural food during the first six months or until such time when *lablab* (complex mat of benthic organisms) and *lumut* (filamentous green algae) growth in the ponds can no longer sustain them. Supplemental feeding with commercial formulated fish pellets (20% protein) at 1-2% body weight was given twice daily. Growth rate of stock from fry stage to a desired culture size was monitored. Stocking density in the ponds also varied according to the initial age of stock, with an optimum density of at least 1,000 juveniles averaging 250 g during the first year.

The broodstocks were transferred to circular maturation cages after six months to one year of pond rearing. The floating maturation cage is 10 m in diameter with a depth of 3 m. Cage design and construction were based on Marte et al. (1984). Each cage was stocked with 250 yearlings. The fish were transported to the cage site with the use of a netted fish barge with bamboo poles as floats or a modified boat-shaped fish cage made of bamboo towed slowly to the cage site. Oxygenated plastic bags and/or holding tanks made of canvas materials supplied with aerators were also used.

The fish were initially fed with commercial pelleted feed (20% protein) at 1.5-2.0% weight until they were three years old after which they were given commercial pelleted crustacean feed (42% protein) at 2-3% of the body weight. Feeding was done twice daily in the morning and afternoon by the broadcast method. When the stocks reached the fifth year, during which spawning was expected, the feeding rate was increased to 5% body weight (Marte et al., unpublished data).

The cages were maintained regularly. This involved: (a) daily inspection to detect tears in the nettings and to remove debris and fouling organisms; (b) periodic rotation of cylindrical styrofoam floats or replacement of rectangular wooden float boxes; (c) repainting of pipe frames with coal tar; and (d) changing of nets as often as needed to protect them from fouling organisms. Fouled nets were sundried, cleaned and repaired. The nets were not changed two or three months before each breeding season to avoid disturbance which may inhibit maturation and spawning.

The physicochemical parameters monitored during the program were temperature, salinity, water transparency, weather and sea conditions. The pertinent data were recorded daily.

Length-weight measurements were taken through semi-annual samplings. A maximum of 10 fish/cage were retrieved by hook and line, then anaesthetized with 2-(phenoxyl) ethanol. Gross morphometric measurements of body weight, total, fork, standard and pre-anal lengths, and body depth were taken. Selected representative samples were dissected quarterly starting in year 4 to determine gonadal development, gonadosomatic indices (GSI) and fecundity for females. Gonad samples which were in the advanced stages were sent to SEAFDEC-AQD for histological examination.

Results and Discussion

Twelve project sites were identified and selected in the different regions of the country. All stations established were maintained for five years with maturation cages as the main holding facility. The locations of these stations are shown in Fig. 1. Of the 12 sites, four were categorized as viable and ideal with the broodstock attaining advanced gonadal stages. These stations are in Alaminos, Pangasinan (Region I); Masinloc, Zambales (Region III); Calape, Bohol (Region VII); and Sta Cruz, Davao del Sur (Region XI). Three of the remaining sites were considered unsuitable because of extensive damage to cages caused by frequent typhoons which necessitated temporary suspension of the project. These stations are being considered for rehabilitation through relocation to sites more protected from typhoons and other adverse weather conditions. The other five stations are maintaining broodstocks which are less than four years old.

Typical annual growth in an ideal station was shown in cage 2, Alaminos site (n = 10): 1981, 26.5 cm total length; 1982, 41.5 cm; 1983, 51.3 cm; 1984, 63.0 cm; and 1985, 66.0 cm.

Sexual maturation was observed in most of the stocks. The maturation data for 1985 are summarized in Table 1. Females with well-developed ovaries were obtained in the later sampling, one female showing apparently regressed gonads. Most of the males with matured gonads were running males. Immature and early maturing fish were obtained mostly in the March sampling. The prevalence of late maturing to mature gonads in fish obtained from Regions I and XI in the August sampling indicates a possible spawning of the stocks in these stations by early 1986. Initial results of fecundity counts indicated a possibility of earlier maturation in the stocks of Region III, i.e., in about three years only. Fecundity was 660,000-1,100,000 eggs/female.

Table 2 shows the ranges of the physicochemical parameters monitored in the sites. Very wide ranges for temperature, salinity and water transparency were observed. The very low values reported for the three parameters were consistent and were observed on days with prolonged heavy rainfall. However, the possibility that significant errors may have been committed cannot be discounted as some of the instruments used were unreliable. The effect of these parameters on the long-range maturation of milkfish broodstock under different ecological conditions should therefore be studied further.

The overall results indicate that milkfish maturation in the Philippines is viable and can be undertaken under different ecological conditions as provided by the different locations of the twelve project stations. However, more in-depth study should be undertaken to develop: (1) more defined ecological requirements; (2) more cost-effective size and design of floating cages; (3) economics of production/milkfish maturation; and (4) in the eventual spawning of the stocks, a very efficient egg-collection method for open maturation systems such as floating cages.

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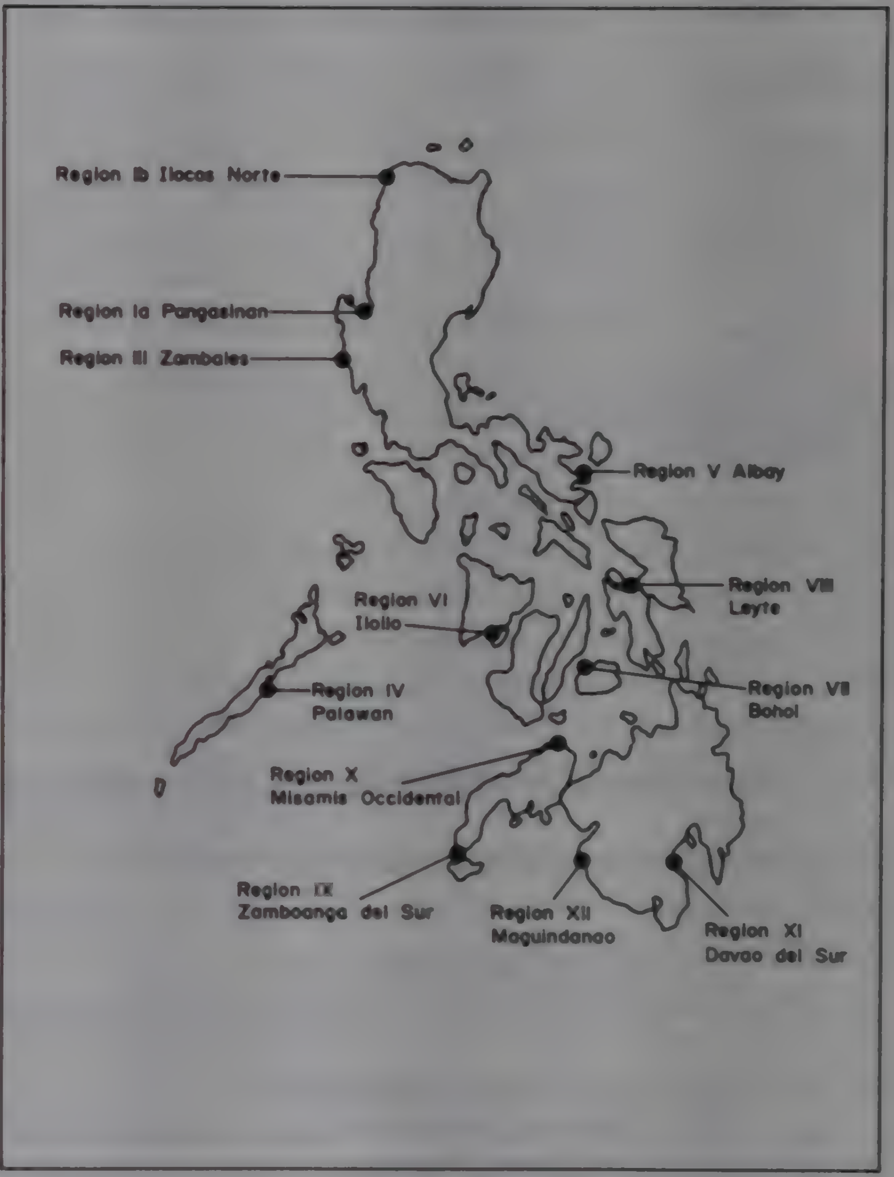

Fig. 1. Sites of the Philippine National Bangus Breeding Program.

Table 1. Gonadal maturation of broodstock in selected stations, December 1985.

		March 1985 sampling												August-November 1985 sampling											
		Age of stock	Immature*			Maturing		Mature		Spent/ regressed			Age of stock	Immature*			Maturing		Mature		Spent/ regressed		
Region/location	Cage no.	(yr/mo)	F	U	M	F	M	F	M	F	M	Total**	(yr/mo)	F	U	M	F	M	F	M	F	M	Total**
Ia — Lucap, Alaminos,	1	4/3	1	—	2	1	1	—	—	—	—	5	4/8	—	—	—	—	3	2	—	—	—	5
Pangasinan	2	4/3	2	—	3	—	—	—	—	—	—	5	4/8	1	—	1	1	—	—	1	1(?)	—	5
	3	4/3	1	—	2	2	—	—	—	—	—	5	4/8	3	1	—	1	—	—	—	—	—	5
	4	4/3	2	—	1	—	—	—	—	—	—	3	4/8	2	1	2	—	—	—	—	—	—	5
	5	3/5	5	—	1	—	—	—	—	—	—	6	3/10	1	—	1	—	2	—	1	—	—	5
	6	3/5	3	—	2	—	—	—	—	—	—	5	3/10	2	3	—	—	—	—	—	—	—	5
	7	3/5	3	—	2	—	—	—	—	—	—	5	3/10	2	3	—	—	—	—	—	—	—	5
Total			17	—	13	3	1	—	—	—	—	34		11	8	4	2	5	2	1	1	—	35
III — Masinloc,	1	4/0	3	—	1	2	—	—	—	—	—	6	4/5	1	—	3	1	—	—	—	—	—	5
Zambales	2	4/9	2	3	—	—	—	—	—	—	—	5	5/2	2	1	1	—	—	—	—	—	—	4
Total			5	3	1	2	—	—	—	—	—	11		3	1	4	1	—	—	—	—	—	9
VII— Calape, Bohol	1	4/8	5	—	—	1	—	—	—	—	—	6	5/1	3	—	1	1	—	—	—	—	—	5
	2	3/8	—	—	—	—	—	—	—	—	—	0	4/0	1	—	1	—	—	—	—	—	—	2
Total			5	—	—	1	—	—	—	—	—	6		4	—	2	1	—	—	—	—	—	7
IX — Sangali, Zamboanga City	1	2/11	1	—	3	1	—	—	—	—	—	5	3/4	—	1	3	—	1	—	—	—	—	5
X — Baliangao, Misamis Occ.	1	2/9	—	—	3	1	—	—	—	—	—	4	3/2	—	—	—	1	—	—	—	—	—	1
XI — Sta. Cruz, Davao del Sur	1	4/2	—	—	—	1	2	—	2	—	—	5	4/7	—	—	—	2	—	1	1	—	—	4
XII— Parang,	1	3/7	1	2	—	—	—	—	—	—	—	3	4/0	3	—	—	—	—	—	—	—	—	3
Maguindanao	2	3/7	—	2	—	—	—	—	—	—	—	2	4/0	1	2	—	—	—	—	—	—	—	3
Total			1	4	—	—	—	—	—	—	—	5		4	2	—	—	—	—	—	—	—	6

*F = female; M = male; U = undertermined.
**Dissected samples only.

Table 2. Range of observed/reported physicochemical parameters in the twelve stations during the five-year maturation period.

	Parameters		
Region/location	Temperature (°C)	Salinity (ppt)	Water transparency (m)
Ia — Lucap, Alaminos, Pangasinan	23-32	19-31	0.5 — 6.0
Ib — Pasuquin, Ilocos Norte	19-34	23-37	1.5 — 6.0
II — San Vicente, Cagayan	—	—	—
III — Masinloc, Zambales	26-32	25-35	1.0 — 10.5
IV — Puerto Princesa, Palawan	20-36	9-34	1.2 — 4.5
V — Bacacay, Albay	—	29-30	2.0 — 3.0
VI — Nueva Valencia, Guimaras, Iloilo	24-32	25-40	1.0 — 5.0
VII — Calape, Bohol	21-32	14-40	2.5 — 11.0
VIII — Babatngon, Leyte	—	—	—
IX — Sangali, Zamboanga City	26-31	18-26	2.5 — 5.0
X — Baliangao, Misamis Occidental	26-34	30-43	0.5 — 7.4
XI — Sta. Cruz, Davao del Sur	25-33	15-29	0.4 — 9.2
XII — Parang, Maguindanao	21-30	29-43	1.0 — 9.2

Effects of Teaseed Cake on Selective Elimination of Finfish in Shrimp Ponds*

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Abstract

Teaseed cake contains 5.2-7.2% saponin, a glucoside that causes hemolysis in organisms. The higher sensitivity of finfish than crustaceans to the glucoside has made it an effective pesticide in shrimp ponds. To develop management techniques for the use of teaseed cake, the effect of dissolved oxygen (DO) and temperature at levels normally found in shrimp ponds on the potency of the toxicant and its rate of degradation when mixed with water were investigated. The experiments were conducted in 20-l plastic tanks, using two species of finfish, *Oreochromis mossambicus* and *Glossogobius giurus*, and two species of crustaceans, *Metapenaeus ensis* and *Penaeus monodon*. The experiments were run on a completely randomized design with three replicate tanks for each treatment. In experiment 1, 15 ppm of teaseed cake was needed to eliminate both species of finfishes within six hours of application. Significant differences in the response of the two species of finfishes were observed. Both species of crustaceans survived concentrations of up to 20 ppm. Results of experiment 2 showed that the decrease of DO levels due to lack of aeration and the increase in water temperature resulting from exposure to sunlight significantly increased the sensitivity of finfish to teaseed cake. Exposure to sunlight for about 12 hours significantly decreased the potency of the glucoside on *O. mossambicus* in another experiment. The change was small and was not observed with *G. giurus*. It is recommended that the water level in shrimp ponds be reduced to one third before application, that teaseed cake be applied in shrimp ponds in minimum dosages towards noon when water temperature is higher and that the water depth be restored after about six hours of application.

Introduction

Predators and competitors adversely affect the growth and survival of shrimps cultured in earthen ponds. The resultant losses increase with increasing intensity of culture of the shrimps. This necessitates the application of a toxicant, which at certain concentrations specifically kills finfish and which is naturally degradable. None of the inorganic pesticides meet the requirement for specificity. Furthermore, these chemicals, particularly the chlorinated hydrocarbons such as endrin, thiodan and DDT remain persistent in the environment, resulting in cumulative effects on other organisms.

Toxicants which are naturally occurring in plants are degradable and finfish can be more sensitive to its toxic properties than crustaceans. Rotenone, the toxin derived from *Derris* sp. was demonstrated to eradicate *O. mossambicus* without affecting the survival of shrimps (Peterson 1976). A similar effect on the undesirable fish in shrimp ponds in Taiwan with teaseed cake has been observed (Terazaki et al. 1980). Teaseed cake, the residue of *Camellia* sp. seeds after oil extraction, contains 5.2-7.2% saponin (Terazaki et al. 1980), a water soluble glucoside which destroys red blood cells.

Teaseed cake may vary in saponin content. Recommended levels for use in eradicating undesirable fish in shrimp ponds are 10-25 ppm of pond water (Cook 1976) or 1.1 ppm of crude saponin which is equivalent to 21 ppm of pond water if the teaseed cake contains 5.2% saponin (Terazaki et al. 1980). It is of great interest for a fish farmer to know the conditions that can make the minimum concentration of teaseed cake effective. Such knowledge can minimize expenses for teaseed cake and energy to pump water to dilute teaseed cake to levels not stressful for shrimps.

A series of studies was conducted to refine the methods of applying teaseed cake in shrimp ponds. Specifically, the studies aimed to determine: the minimum concentration of teaseed cake that would effect a selective elimination of finfish within three to six hours; the differences in the response of *O. mossambicus* and *Glossogobius giurus* to teaseed cake; the effect of temperature and dissolved oxygen levels, within the ranges normally found in ponds, on the potency of teaseed cake as a toxicant; and whether substantial degradation of teaseed cake can occur within 24 hours.

Materials and Methods

The experiments were conducted in 20-l round plastic tanks, using three replicate tanks for each treatment and two pieces each of the following finfish and shrimps in each tank: *O. mossambicus*, *G. giurus*, *Metapenaeus ensis* and *Penaeus monodon*. Finfish of 10.6-18.6 g and shrimps of 2.7-5.6 g were stocked in the tanks containing water at 21-31 ppt salinity and exposed to the various treatments in completely randomized design. Teaseed cake used in the experiments was imported from Taiwan.

In experiment 1, the concentration of teaseed cake that would effectively eliminate finfish within three to six hours, without adversely affecting the survival of shrimps was determined. The test species were exposed to 2.5, 5, 10, 15 and 20 ppm of teaseed cake for 48 hours. Mortalities were monitored every hour for the first eight hours and at the 24th and 48th hour. Results were expressed at LT_{50} or LT_{100}, representing the time required for half or all the test animals to be killed, respectively.

In experiment 2, the effect of temperature and DO on the potency of teaseed cake was evaluated at 2.5-15 ppm concentrations in tanks, with or without exposure to sunlight or aeration in a 2 x 2 factorial design. Sunlight was used to raise temperature to simulate the temperature range observed in shrimp ponds. A marginal concentration of 2.5 ppm was used to better evaluate the extent of the effects of the temperature and DO ranges used. Water temperature and DO levels were monitored every two hours and mortality every hour. Teaseed cake was applied at 9 a.m. The test species were exposed to the various experimental conditions for six hours.

To determine whether significant degradation of teaseed cake occurred within 24 hours, tanks containing 15 ppm of the toxicant were exposed for various durations (0, 4, 12, or 24 hours) prior to stocking in experiment 3. Five of each species of finfish and shrimps were stocked in each tank. Water temperature and DO levels were monitored every two hours and mortality every hour. Aeration was provided in all tanks.

Results

Table 1 shows that the survival of the shrimps was not affected by the range of concentration of teaseed cake used during the period of observation. Both species tolerated up to 20 ppm of teaseed cake.

Significant differences in the response of the finfish were observed. *O. mossambicus* was more sensitive to the toxicant, with an LT_{100} of 3 hr compared to an LT_{100} of 7 hr for *G. giurus* when exposed to 10 ppm teaseed cake (Table 1). The survival of *G. giurus* was, however, higher with longer exposure to concentrations below 10 ppm. Fifteen ppm was needed to completely eradicate this species within 6 hours: thus, this concentration was recommended as the lethal dosage.

Similar results were observed in a study conducted in 40-m^2 earthen ponds. Neither *O. mossambicus* nor *G. giurus* survived after exposure to 10 ppm teaseed cake after 48 hours.

Tanks exposed to sunlight increased steadily in temperature from mean of 29°C at 8 a.m. to 35°C at 12 noon. Temperature decreased only by 1°C thereafter (Fig. 1). Tanks not exposed to sunlight maintained a temperature range between 27 and 29.2°C.

Tanks provided with aeration maintained DO levels above 2 ppm throughout the experiment (Fig. 2). DO levels in tanks not aerated steadily decreased from an average of 3.6 ppm at 8 a.m. to less than 1 ppm at 2 p.m.

Table 2 shows that both species of finfish did not survive a three-hour exposure to teaseed cake at 15 ppm. Consistent with experiment 1, *O. mossambicus* responded faster to teaseed cake at 15 ppm, but fewer *G. giurus* survived a longer exposure (six hours) at 2.5 ppm.

Higher temperature resulted in significantly lower survival of *O. mossambicus* during hour 1 at 15 ppm ($P < 0.10$) and a signicantly lower survival ($P < 0.01$) at high temperature after a three-hour exposure to 2.5 ppm (Table 2). Likewise, aeration, which resulted in an average difference of 2.5 ppm in DO levels, effected significantly higher ($P < 0.05$) survival of *O. mossambicus* at hour 1 at 15 ppm and of both species at hour 6 at 2.5 ppm. Higher survival for *G. giurus* exposed to aeration at 15 ppm of teaseed cake was also observed at hour 1.

Consistent with experiments 1 and 2, *O. mossambicus* responded faster to exposure to teaseed cake at 15 ppm than *G. giurus* (Table 3). The teaseed cake was exposed in the tank with water for varying periods (0, 4, 12 and 24 hours) prior to stocking of the test species. After two hours of exposure, *O. mossambicus* treated with teaseed cake exposed earlier by 12 and 24 hours, had significantly ($P < 0.05$) higher survival than those treated with teaseed cake exposed earlier to four hours or less. No such difference was observed with the slower responding *G. giurus*.

Discussion

The rate of response of finfish to teaseed cake demonstrates that *O. mossambicus* is more sensitive to saponin than *G. giurus*. At 2.5 ppm, however, longer exposure resulted in more mortalities for *G. giurus*. On the other hand, Terazaki et al. (1980) observed that *O. mossambicus* was more resistant to saponin than *Eleutheronema tetradactylum*, *Mugil tade* and *Scatophagus argus*. Organisms generally exhibit distinct differences in sensitivity to toxicants, both at the individual and species level (Khan and Bederka 1973). Temperature and DO levels affect the response of the organims to toxicants. The response generally increases at higher temperature as a result of increased rate of metabolism. DO levels, when limiting, can cause stress and result in increased sensitivity of organisms to toxicants.

This study showed that both high temperature and low DO can result in increased sensitivity of the finfish to the toxicant. In practice, the higher range of temperature (35ºC) is normally observed in shrimp ponds at about noontime when DO levels are relatively high. Moreover, the low range of DO is not observed by those who provide aeration by paddlewheels.

In experiment 1, 15 ppm was defined as the recommended dosage for teaseed cake, being the level which resulted in the complete eradication of the finfish within six hours. The results of experiment 2 suggest that in practice, 10 ppm concentration can be used with the same effects if the toxicant is applied about noontime when the temperature is highest. This can result in tremendous savings, not only by 33% of the cost of teaseed cake but also in the reduction of energy required to pump water for flushing the pond to bring down the levels of saponin to that not stressful for shrimps. In a field trial, shrimps were observed not to feed normally when 15 ppm teaseed cake was applied. They appeared to return to normal feeding after the concentration was reduced to about 3 ppm. Experiment 3 demonstrated that although the degradation of teaseed cake may be significant after a 12-hour exposure to water, the rate of degradation is slow. This suggests the need to dilute pond water as soon as possible, so that shrimp production will not be adversely affected by the application of teaseed cake. From the series of experiments conducted, it is recommended that the water level in shrimp ponds be reduced to one third before application, that teaseed cake be applied after 10 a.m. on a sunny day and that water depth be restored after about six hours of application.

The dosages reported here need not be absolutely followed. Terazaki et al. (1980) reported different levels of saponin from teaseed cake from different sources, and varying responses to saponin resulting from differences in fish sizes and salinity levels. Preliminary assays of different batches of teaseed cake are therefore recommended prior to use in shrimp ponds.

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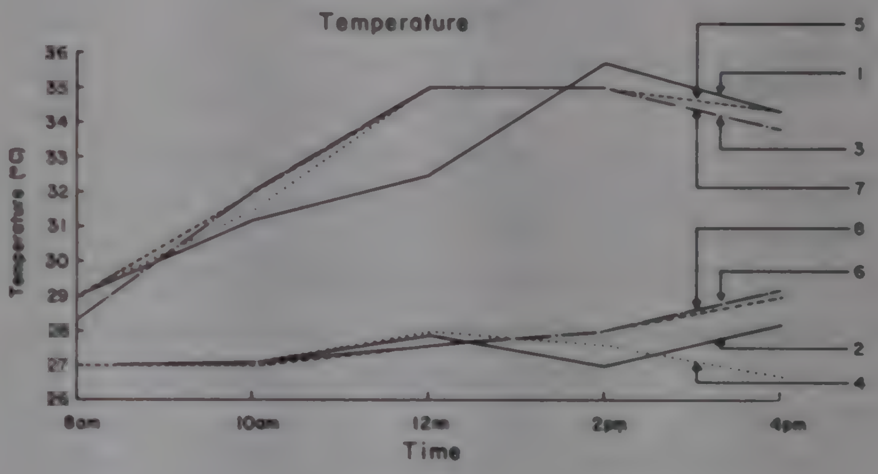

Fig. 1. Temperature (°C) among different treatments. Numbers represent the same treatments as presented in Table 2.

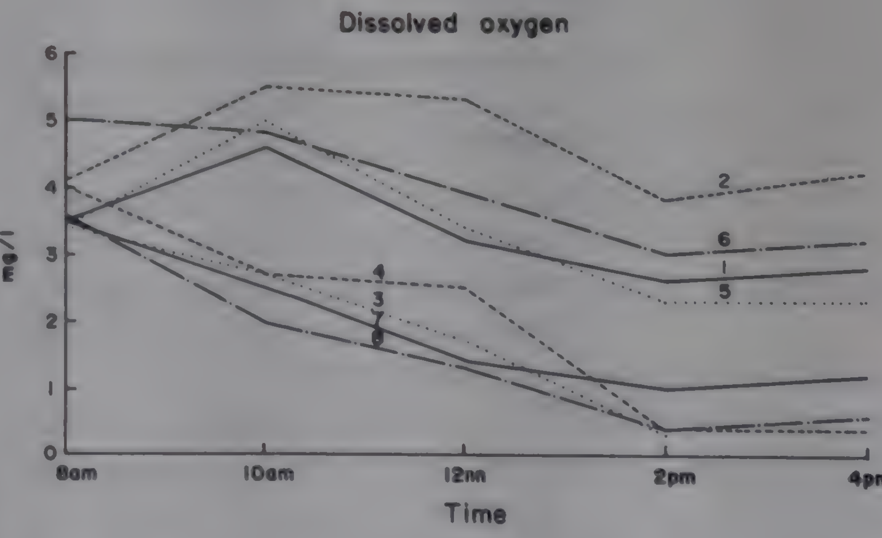

Fig. 2. Dissolved oxygen (mg/l) among different treatments. Numbers represent the same treatments as presented in Table 2.

Table 1. LT_{50} and LT_{100} of finfishes and shrimps at different dosages of teaseed cake (Experiment 1).

Species	Dosage of teaseed cake (ppm)	LT_{50} (hr)	LT_{100} (hr)
O. mossambicus	2.5	*	*
	5.0	3.0	6
	10.0	2.5	3
	15.0	2.0	3
	20.0	1.5	2
G. giurus	2.5	6.0	> 48
	5.0	3.0	8
	10.0	5.0	7
	15.0	4.0	6
	20.0	3.5	6
M. ensis	2.5	*	*
	5.0	*	*
	10.0	*	*
	15.0	*	*
	20.0	*	*
P. monodon	2.5	*	*
	5.0	*	*
	10.0	*	*
	15.0	*	*
	20.0	*	*

*Not observed within 48 hr.

Table 2. Effect of temperature and dissolved oxygen on the survival of *O. mossambicus* and *G. giurus* at different concentrations of teaseed cake (Experiment 2).

	% Survival[1] Hr							
Treatment	1	2	3	6	1	2	3	6
	O. mossambicus				*G. giurus*			
I – 15 ppm, aerated, high temp	17	17	0	0	50	17	0	0
II – 15 ppm, aerated, low temp	100	0	0	0	67	33	0	0
III – 15 ppm, not aerated, high temp	0	0	0	0	17	17	0	0
IV – 15 ppm, not aerated, low	0	0	0	0	33	33	0	0
V – 2.5 ppm, aerated, high temp	100	100	83	83	83	33	17	17
VI – 2.5 ppm, aerated, low temp	100	100	83	83	83	83	83	50
VII – 2.5 ppm, not aerated, high temp	100	67	33	0	67	33	0	0
VIII – 2.5 ppm, not aerated, low temp	100	100	100	67	67	33	33	17
Analysis of variance	p values[2]							
Concentration	.01	.01	.01	.01	.05	.10	.01	.01
Aeration	.01	.05	NS	.05	NS	NS	.05	.05
Temperature	.01	NS	.05	NS	NS	.10	.01	NS
Concentration x Aeration	.01	NS	NS	.05	NS	NS	.05	.05
Concentration x Temperature	.01	.05	.05	NS	NS	NS	.01	NS
Aeration x Temperature	.01	.05	.05	NS	NS	NS	NS	NS
Concentration x Aeration x Temperature	.01	NS	.05	NS	NS	NS	NS	NS

[1] Values represent the mean of 3 replicates.

[2] Source of variation indicated as significant ($P < 0.10$, $P < 0.05$, $P < 0.01$).

Table 3. Effect of exposing *O. mossambicus* and *G. giurus* to teaseed cake for various durations before stocking (Experiment 3).

Exposure time of teaseed cake (hr)	% Survival					
	1	2	3	4	5	6
O. mossambicus						
0	100	7	0	0	0	0
4	7	7	0	0	0	0
12	80	33	0	0	0	0
24	100	20	0	0	0	0
G. giurus						
0	93	93	93	93	67	40
4	100	100	80	53	27	27
12	100	87	87	80	47	40
24	100	100	100	100	67	47

Water Quality Dynamics in Brackishwater Shrimp Ponds with Artificial Aeration and Circulation[*]

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Abstract

Water aeration and circulation using paddlewheel aerator and motor-driven propeller blades, respectively, were maintained under four different diurnal regimes to test their effects on water quality and production of *Penaeus monodon*. Shrimp with an average weight of 0.03 gm were stocked at a density of 33 individuals/m^2 in four 0.05-ha earthen ponds. The four treatments were: (1) continuous aeration (2) nighttime aeration (3) nighttime aeration and daytime circulation and (4) no aeration or circulation. Ammonia nitrogen (NH_3-N), dissolved oxygen, temperature and shirmp growth were monitored during a culture period of 10 weeks. Ammonia nitrogen concentration did not differ significantly under the various aeration and circulation treatments. Dissolved oxygen and temperature stratification was least evident in treatments 1 and 3. Nighttime dissolved oxygen levels were significantly lower in treatments 1 and 4. The average size of shrimp at harvest was significantly higher in treatments 1 and 3. These findings taken together indicated that there was no clear benefit from continuous aeration. Rather, a combination of nighttime aeration, when needed, and daytime circulation could result in the greatest water quality benefits with the least energy consumption.

Introduction

The effects of different combinations of continuous and intermittent artificial circulation/aeration on water quality and shrimp growth are not well documented. Many types of artificial aeration and circulation are used in intensified shrimp farming, but their respective impacts on such factors as dissolved oxygen concentrations and ammonia are not well known. For example, many shrimp farmers use paddlewheel aeration during nighttime only to prevent critically low dissolved oxygen concentration, while other farmers use continuous aeration after the shrimp reach a certain biomass.

Continuous aeration appears at first to be self-defeating as oxygen is typically supersaturated at midday. Such aeration strips oxygen from the water column during the day until equilibrium is reached at 100% saturation. The benefits of daytime aeration, if there are any, may be due to other factors as oxygen is not the only dissolved gas affected by aeration. Ammonia gas, or un-ionized ammonia, is also affected and should be removed from the water by aeration. This is particularly true during periods of elevated pH and water temperature. Even without aeration a substantial amount of ammonia is degassed from eutropic waters.

Conditions of high pH and temperature are found at midday, especially when oxygen values are greatest. Thus, the time of the day when aeration can remove the greatest amount of dissolved ammonia coincides with the time of the day when aeration can also remove the greatest amount of oxygen. The benefits of ammonia removal could outweigh the negative effect of oxygen removal. If this is the case, then continuous aeration under intensive culture conditions could be a better water quality management strategy than nighttime aeration only.

Fast et al. (1983) found that artificial circulation, without aeration, caused a redistribution of oxygen throughout the water column, but did not materially affect the exchange of oxygen between the pond water and the atmosphere. This should result in the conservation of dissolved oxygen within the pond. At the same time, it might also reduce the loss rate of ammonia, or at least result in less ammonia loss compared with aeration, or even compared with an undisturbed condition.

Because of these unknown and potentially opposing influences of artificial aeration on water quality, this experiment was designed to determine the respective effects of aeration and circulation on dissolved oxygen, temperature and ammonia concentration.

Materials and Methods

Four 500-m^2 ponds were treated as follows: treatment 1. continuous aeration, 24 hr/day; treatment 2. nighttime aeration only from 2100-0600 hr; treatment 3. nighttime aeration from 2100-0600 hr and daytime circulation from 0600-2100 hr; treatment 4. control with no aeration or circulation.

The aerator consisted of a 0.5 hp paddlewheel similar in design to those used in Taiwan. The two paddles 0.6 m dia revolved at 138 rpm. The circulator was a 1/8 hp similar in design to the PC-11 described by Fast et al. (1983). It consisted of 40-cm fan blade which revolved at 86 rpm.

Each pond was dried before the experiment. No lime or chicken manure was added as the shrimp were given a complete artificial diet. The ponds were then filled with water to 1.0 m and stocked with *Penaeus monodon* on 25 February 1985 at 33/m^2. Their average weight was 0.03 g. Every two weeks 80% of the water was changed, the pond depth maintained at 1 m.

Oxygen and temperature were measured 5 times/week at 0400, 1000, 1400 and 1700 hr daily with a YSI model 51B oxygen meter. The pH was also measured 5 times/week with a Corning pH meter. Chlorophyll was measured twice/week by acetone extraction as described by Strickland and Parsons (1976). Ammonia was measured twice/week by the phenol hypochloride and ascorbic acid methods described by Strickland and Parsons (1976).

Shrimp were sampled and weighed every two weeks. After 63 days, on 3 March 1985, the ponds were drained and the total weight of the shrimp measured along with a subsample of individual weights.

Results and Discussion

Average whole pond temperatures for all dates were lowest at 0400 hr and highest at 1400 hr. The averages for all the ponds were 27.3 and 30.4°C, respectively (Fig. 1 and Table 1). This represents a 3.1°C temperature fluctuation per day. On the average, the control pond was slightly warmer than the treatment ponds. The reason for this is unknown, but it could be due to a high water flushing rate caused by uncontrolled leaks.

The differences in temperature between the pond surface and the bottom water is a measure of the degrees of thermal stratification. The temperature differences between treatments at 0400 hr showed no significant differences (Table 2). This reflects not only the influence of the aerators in the three treatment ponds during the night, but also the effects of diurnal cooling and mixing which occurred each night. By 1000 hr there were no significant differences between surface and bottom temperatures in treatment 1, while in all the other treatments there were significant temperature differences between the surface and the bottom (Table 2). In treatment 3 there was a significant temperature difference, although at a lower probability level ($P = 0.05$).

By 1400 and 1700 hr there was a highly significant difference in surface and bottom temperatures in treatments 2 and 4 but none in treatments 1 and 3.

These results substantiate earlier findings by Fast et al. (1983) in Hawaii where artificial circulation resulted in nearly uniform water temperatures throughout the pond, from surface to bottom, during the day in contrast with the substantial thermal stratification without circulation. Mixing raised the temperature at the pond bottom, resulting in evenness.

Average dissolved oxygen (DO) concentrations in all ponds for all dates closely paralleled the pattern for temperature. The lowest values for all ponds averaged 4.4 mg/l at 0400 hr and 9.6 mg/l at 1400 and 1700 hr (Table 3 and Fig. #2). All the ponds aerated during the night had higher DO at 0400 hr than the control. Treatment 1 with daytime aeration had lower DO than treatments 2 and 3 with nighttime aerations (8.8 vs. 9.7 mg/l). These results follow the hypothesis that daytime aeration will result in oxygen loss from those ponds that reached supersaturation during the day as artificial aeration causes oxygen concentrations to change towards 100% saturation regardless of whether the water is undersaturated or supersaturated.

Also of interest is the observation in treatment 3 which has daytime oxygen concentration similar to those of treatments 2 and 4. This confirms that daytime circulation does not materially effect oxygen exchange between the atmosphere and water, but only eliminates thermal stratification. Fast et al. (1983) also observed elevated daytime bottom oxygen and temperature during artificial circulation.

With the exception of treatment 1, the average differences in oxygen concentrations between the surface and the bottom follow exactly the same trend as did the surface/bottom temperatures (Table 4). We have no explanation for this significant difference.

The greatest significant difference between surface and bottom oxygen values occurred at 1400 and 1700 hr. There was no significant difference between surface and bottom oxygen concentrations in the aerated/circulated ponds at these times, but non-aerated/circulated ponds had significant stratification. These results also follow those reported by Fast et al. (1983) where non-mixing often resulted in strong oxygen stratification and lower oxygen concentrations at the pond bottom. The exception was when wind velocities exceeded about 20 km/hr, at which time even the ponds which were not artificially aerated/circulated had nearly uniform oxygen concentrations.

Whole pond average pH values were always lowest at 0400 hr at 8.0, and greatest at 1700 hr at 8.4. There did not appear to be much difference between treatments.

Whole pond average ammonia concentrations were lowest in the pond with continuous aeration, and next lowest in the pond with nighttime aeration and daytime circulation. These values were 0.044 and 0.055 mg/l, respectively, compared with 0.065 and 0.061 mg/l for the ponds which were not aerated/circulated during the day (Table 5). Although these values are consistent with the hypothesis that daytime aeration/circulation will cause a greater atmospheric loss of ammonia than the non-circulated condition, the difference was not statistically significant ($P > 0.05$). These findings, while somewhat inconclusive, are difficult to assess strictly since there was a great deal of variability in our ammonia data. This variability may be due to the dynamic nature of ammonia, as well as analytical limitations at these relatively low concentrations. It can be concluded, however, that aeration/circulation under the test conditions used did not cause a significant reduction in ammonia concentrations.

Chlorophyll A values were highest in the pond with continuous aeration and lowest in the control. The values were 99.02 and 53.05 mg/1 respectively (Table 6). Even with this range, analysis of variance revealed no significant difference between treatments, perhaps due to the large variation of concentration between sampling days.

Average shrimp size at harvest ranged from 2.1 g in the control to 2.8 g in treatment 1 and in treatment 2 (Table 7). There was no significant difference between treatments 2 and 3 or between the control and treatment 3. These results indicate that aeration/circulation contribute to faster growth compared to the undisturbed condition, but these are not conclusions because of lack of replication and the insignificant differences between the control and treatment 3.

Acknowledgements

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Table 1. Whole pond average water temperature (°C) for each treatment, 4x day.

	Sampling period (hr)			
Treatment	0400	1000	1400	1700
1	27.3	28.2	30.2	30.3
2	27.2	28.2	30.2	30.3
3	27.2	28.3	30.4	30.3
4	27.5	28.8	30.9	30.8

Table 2. Average temperature (°C) of pond surface and bottom at sampling times.

Sampling period	Treatment	F-test
0400 hr	1	n.s.
	2	n.s.
	3	n.s.
	4	n.s.
1000 hr	1	n.s.
	2	**
	3	*
	4	**
1400 hr	1	n.s.
	2	**
	3	n.s.
	4	**
1700 hr	1	n.s.
	2	**
	3	n.s.
	4	**

n.s. = not significant; ** = $P < .01$; * = $.01 < P < .05$.

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*Contribution Number CRSP 86.4

Table 3. Average dissolved oxygen (ppm) for each treatment 4x day.

	Sampling period (hr)			
Treatment	0400	1000	1400	1700
1	4.4	7.0	9.0	8.8
2	4.5	6.9	9.7	10.0
3	4.8	7.1	9.8	10.0
4	3.7	6.1	9.5	9.6

Table 4. Average dissolved oxygen (ppm) at pond surface and bottom at sampling times.

Sampling period	Treatment	F-test
0400 hr	1	**
	2	n.s.
	3	n.s.
	4	n.s.
1000 hr	1	n.s.
	2	**
	3	*
	4	**
1400 hr	1	n.s.
	2	**
	3	n.s.
	4	*
1700 hr	1	n.s.
	2	**
	3	n.s.
	4	**

n.s. = not significant; ** = $P < .01$; * = $.01 < P < .05$.

Table 5. Average ammonia concentrations.

Treatment	mg/l
1	0.044
2	0.065
3	0.055
4	0.061

F computed = 0.6073 < F (0.05; 3.39 d.f.) = 2.89 n.s.

Table 6. Average chlorophyll A concentrations.

Treatment	mg/l
1	99.02
2	84.30
3	63.02
4	53.05

F computed = 2.083 < F (.05; 3.39 d.f.) = 2.89 n.s.

Table 7. Average weight of shrimps at harvest.

Treatment	gm	P < .05	P < .01
1	2.82	a	a
2	2.79	a	ab
3	2.36	b	abc
4	2.09	b	c

F computed = 7.026 < F (.01; 3,396) = 3.78 **.

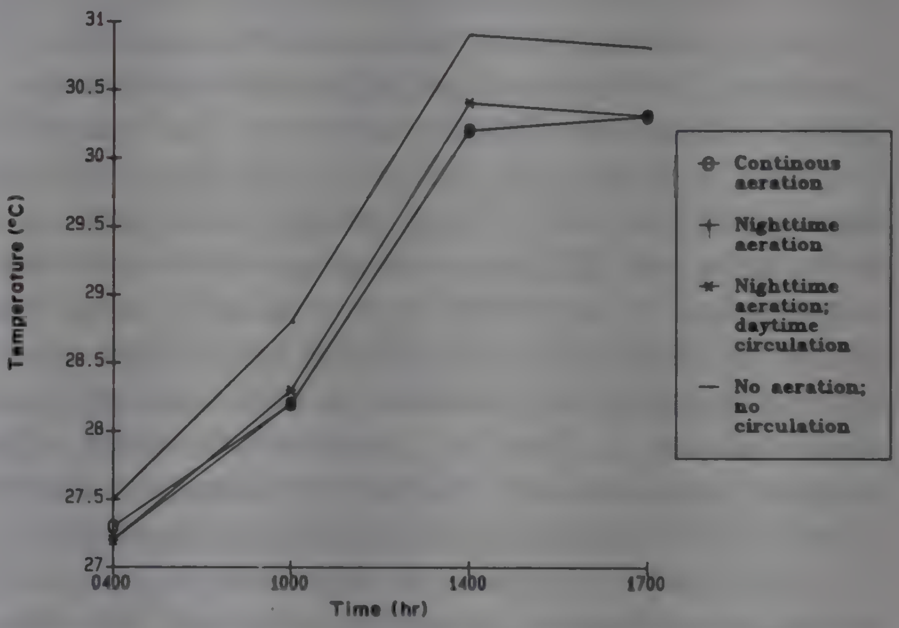

Fig. 1. Average temperature level at different sampling periods.

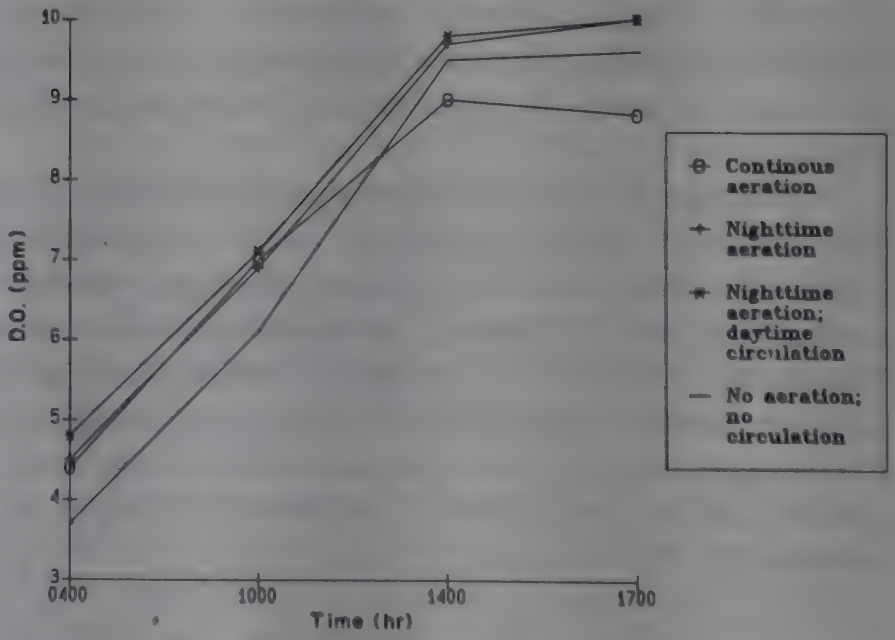

Fig. 2. Average dissolved oxygen level at different sampling periods.

Seasonal Ponds - A Potential Source of Fish Seed in Rajasthan, India

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Abstract

Fish seed is the most important component for fish culture in Rajasthan, India. For stocking the 300,000 ha of freshwater resources in the state, the total requirement is 325 x 10^6 fish seed. Out of the total available 120 x 10^6 fish seed, 80% is obtained from seasonal ponds, known as dry bundhs. The technique can be gainfully employed for selective breeding and hybridization of Indian and Chinese carps. It is concluded that dry bundh breeding along with a modern hatchery system can augment fish seed production in the state.

Introduction

The freshwater resources of Rajasthan State are estimated to be about 300,000 ha of reservoirs, lakes, tanks, ponds, borrow-pits and low-lying water masses. The present need is to exploit the water resources to meet the fish requirement of the people. These resources, however, require restocking almost every year. The fish seed could be obtained by collection from natural sources, permanent ponds, dry bundhs and hypophysation. The collection of fish seed from natural sources is neither dependable nor profitable. By hypophysation the adult fish is sacrificed and the prospective brooders are lost.

The breeding of fish in dry bundhs was prevalent in West Bengal and was first noticed in 1882 by a private pisciculturist (Manu Tali) who successfully collected spawn from 'Corabat Bundh' in Bankura district (West Bengal) in India. The systematic dry bundh breeding of Indian major carps started in 1926 in the same district.

Dry bundh breeding was first initiated in Rajasthan as early as 1966 in the 'Amer Ki Talai'. Since then a thorough survey was conducted in the state and about 139 dry bundhs were identified, most of them surrounded by the Aravli hills.

Bundh Resources

A typical 'dry bundh' is a seasonal type pond having a vast catchment area in red laterite land with a sluice gate. A waste weir facilitates the breeding operations in the dry bundh. The surrounding land has excellent soil (bhata) and provides an ideal catchment area and quick filling of the dry bundh even with a short duration of rain. It also provides quick and easy drainage.

The catchment area of the dry bundh is 1-16 km^2. The area when full is 100-400 ha and the permanent storage is 2-10 ha 1-m deep. Trees, rocks and shrubs are completely absent in the permanent storage area. Most of the dry bundhs are located along roads and are easily approachable even during the rains.

The soil of a suitable dry bundh is black clay mixed with loam. The slopes are mostly 0-8% with slight to moderate erosion features. The water-holding capacity of soils of the selected bundhs is congenial to fish breeding.

The basins of the selected dry bundhs, being agricultural fields, are congenial to breeding of major carps as farmers utilize the inorganic and organic fertilizers. The pH is favorable and nutrients are abundant, evident from the good growth of fish seed in the bundh. Turbidity is caused by heavy rains, various grades of humus, silt, organic detritus, coloidal water and other kinds of materials. All these carried to the bundh by rains make breeding successful.

There are 700 bodies of water in the state. The general practice is to auction these water resources, but hardly one-third of them are auctioned annually. They are classified as A-class (more than 200 ha); B-class (between 8 and 200 ha) and C-class (less than 80 ha). Nearly 50 such waters are included in A-class, 90 in B-class and 560 in C-class. At present, the A-class waters provide Rs 5 x 10^6; B-class, Rs 4.5 x 10^6 and C-class, 5.6 x 10^6. After development revenue could reach Rs 422.2 x 10^6 if the fish are sold at Rs 500/quintal.

Seed Requirements

Three types of culture have been identified in Rajasthan for seed stocking: intensive, traditional and reservoir culture. The water area included in the first category is 0.04 x 10^6 ha (C-class), 0.14 x 10^6 ha in the second category (B-class) and 0.12 x 10^6 ha in the third category (A-class). Stocking is done at the rate of 5,000 fingerlings/ha in the first category, 700 in the second category and 210 in the third category. The total requirement of fingerlings is 200, 98 and 25 x 10^6, respectively. Nearly 325 x 10^6 fingerlings would thus be needed for total stocking.

In 1978, the total Rajasthan fish production was 12,500 t (Petr 1983). In culture, the average rate of fish production would average 1,000 kg/ha in intensive culture, 300 kg/ha in traditional culture and 20 kg/ha in reservoirs. Thus, fish production could be about 40,000 t by intensive culture, 42,000 t through traditional culture and 2,400 t in reservoirs, a total of about 84,400 t.

For the overall production of 325 x 10^6 fingerlings, approximately 760 x 10^6 fry are required with an allowance for 46% mortality. Of the fingerling requirements, 25 x 10^6 would come from natural collection; 200 x 10^6 from dry bundhs and 100 x 10^6 from induced breeding. A rearing space of 76 ha as nursery for fish seed at the rate of 10 x 10^6/ha would be required. A 250 ha rearing space at the rate of 1 x 10^6 fry/ha would be needed to raise 325 x 10^6 fingerlings, taking into consideration 76 ha nursing area for rearing fingerlings.

Carp Breeding in Dry Bundhs

A sufficient number of breeders of major carps, *Catla catla, Cirrhina mrigala* and *Labeo rohita* is stocked in the bundh with male and female ratio of 2:1 or 3:2. A fisherman is posted to watch over the breeding of fish. Breeding takes place in the dry bundh after heavy to moderate rain. During rainy days the temperature varies between 24 and 31°C. Dubey and Tuli (1961) observed breeding in major carps at various depths of water with varying flow or even in standing water. Soft and clayey substratum with water temperature of 26-33°C and pH 7.2-8.2 was found in these tests suitable for breeding. Before stocking, the bundh bottom should be cleared of stones, wood and other materials.

The rate of stocking of mature fish for breeding is 500-1,000 kg/ha which produce 100,000 eggs/kg of body weight of fish. From a hectare, nearly 50 x 10^6 eggs can be produced. The breeding starts after or on the second stocking of bundhs. At first, smaller mature fish are stimulated and migrate to the shallower areas in the bundh to spawn. The bigger fish spawn next in the same area. Spawning occurs over hard or sandy or muddy soil and even on rocky embankments.

Hatching takes place in the same dry bundh; there is no separate facility for hatching of eggs. The breeding is ascertained only after sampling the water with mosquito netting.

The advantages of dry bundh breeding are that a 100% pure seed of desirable fish is obtained. In dry bundhs the breeding is certain even during local rains. In these bundhs successive seed crops can be harvested in the same season by successive drainage, removing the previous fish and the remaining seed and by introducing fresh sets of mature fish.

Discussion

The reservoirs, lakes, tanks and ponds of Rajasthan State constitute about one-tenth of the fishery resources of India.

Fish farms in Rajasthan have inadequate breeding and rearing technology. Low-investment technology - constructing dry bundhs with sluice gates - would seem attractive.

Poor recovery and low survival rate of fingerlings in dry bundh breeding have been observed. Survival could be improved by adopting new hatching techniques and hybridization of Indian and Chinese carps. Seed production in this manner would be less capital-intensive and could be taken up as a commercially viable scheme with institutional financing.

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Milkfish Pen Culture in Sri Lankan Lagoons

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SIRIWARDENA, P.P.G.S.N. 1986. Milkfish pen culture in Sri Lankan lagoons, p. 89-92. *In* J.L. Maclean, L.B. Dizon and L.V. Hosillos (eds.) The First Asian Fisheries Forum. Asian Fisheries Society, Manila, Philippines.

Abstract

This paper records preliminary observations on milkfish *(Chanos chanos)* pen culture in the Puttalam lagoon on the west coast of Sri Lanka. The experiments were carried out in 0.25-ha rectangular fishpens made of polypropylene nets of 2.5-cm stretched mesh and bamboo pole frameworks. The extrapolated production was 1,600 kg/ha of two cycles. At a stocking rate of 10,000/ha, average growth rates were 0.6 g/day and 0.54 g/day in trials with and without supplementary feeding, respectively. The specific growth rates showed no significant differences. A low-cost feed of 20% protein (rice bran 8 fishmeal 2) was used. The von Bertalanffy parameters estimated were: K = 0.925/year and L_∞= 47.89 cm in trials with supplementary feeding and K = 0.807/year and L_∞ = 50.61 cm in trials without supplementary feeding.

Introduction

Most of the current aquaculture practices in South and Southeast Asia are land based and there is growing concern that land near water sources has become limited (Guerrero and Soesanto 1982). The water-based systems of pen and cage culture have become important towards increasing fish production to meet the annual protein needs.

The culture of milkfish *(Chanos chanos)* in brackishwater ponds is an age-old practice in tropical areas of the Indo-Pacific region (Baliao 1983). Pen culture has been initiated in 1983 in Sri Lanka with a view to utilizing large areas of shallow brackishwater lagoons for production.

Production rates of milkfish raised in pens in the Philippines have been reported by Delmendo and Gedney (1974, 1976), Baguilat (1979), Nicolas and Librero (1979), Librero and Nicholas (1982) and Mane (1982). Chidambaran and Unny (1946) studied the growth of milkfish in seawater, brackishwater and freshwater. Records on the growth and survival of milkfish raised in brackishwater ponds have been given by Tampi (1960), Eldani and Primavera (1981) and Villaluz and Unggui (1983). This paper records some preliminary observations on experimental milkfish pen culture trials during 1984 and 1985.

Materials and Methods

The experimental culture site was in Puttalam Lagoon (Fig. 1). The lagoon has a surface area of about 237 km^2. Perera and Siriwardena (1982) reported that the highest tidal amplitude recorded was 83 cm at Kalpitiya and the lowest was 69 cm at Ethalai.

Indrasena (1984) found that the amount of chlorophyll varied from 1 to 7 mg/l and primary production and phosphate, 0.05 to 0.4 mg c/l/hr and 0.34 to 1.66 mg-atm/l respectively (Indrasena, unpublished data). Durairatnam (1963) noted that there was more than one phytoplankton maximum in a year. The bulk of phytoplankton occurred from May to September during the southwest monsoon due to the cumulative effect of several species and two outbursts of diatoms in June and October. Blue-green algae belonging to the Cyanophyceae abound in January, February, March and November (Durairatnam 1963).

Temperature, salinity, pH and dissolved oxygen were monitored and rainfall and wind speed data (shown in Figs. 2 and 3) were gathered from the Meteorological Department of Sri Lanka.

The pens were made of a 2.5-cm stretched mesh polypropylene net and supporting bamboo framework. The bottom edge of the net enclosure, embedded about 0.5 m in the mud with metal sinkers of 15 kg at 1-m intervals, was held above water by the framework which was made up of vertical bamboo poles arranged at 2.5-m intervals to form a square configuration. Support to the framework was improved by two side bracings at 5-m intervals and by horizontal braces.

The nursery compartments were square and made up 25% of the grow-out pen. The nursery netting was of 5-mm mesh but the framework was similar to that of the grow-out pen without the side bracings.

Two fishpens, each measuring 0.25 ha, were used to observe growth rate, production and survival rate of milkfish. Fingerlings were obtained from the nursery pens where the fry were reared to fingerlings. Both pens were stocked at a rate of 10,000/ha.

Prior to stocking, the pen area was cleaned to remove unwanted species by using a drag net and trammel nets. Fish stocked in pen no. 1 were fed with a low-cost feed of 20% protein consisting of rice bran and fishmeal used at a ratio of 8:2. The manually mixed feed was given daily at 3% of the body weight. Fish in pen no. 2 were not fed.

From each pen a sample of 150-200 fish was taken monthly for total length and weight measurements. After the culture period, the fish were harvested with trammel nets and drag nets.

Results

Tables 1 and 2 show the experimental conditions and growth of fish. Calculated von Bertalanffy parameters are tabulated in Table 3.

There was a significant difference in growth rates of milkfish in the two culture systems (Fig. 4) but the specific growth rates showed no significant difference. Instantaneous rates of mortality were found to be 0.794/year and 0.811/year in culture systems with and without supplementary feeding, respectively.

Discussion

Delmendo and Gedney (1974, 1976) recorded that the annual production of milkfish during the early operations of pens in Laguna de Bay, Philippines, was at least 1,500 kg/ha and average weight of fish was 350 g after five to six months culture. Baguilat (1979) stated that in Laguna de Bay alone about 5,000 ha of fishpens produced an average of 5,000 kg/ha/year. Librero and Nicolas (1982) recorded that a fishpen in Laguna de Bay yielded 3,798 kg/ha/year. This shows that production rate improved with the gaining of experience and resolving problems that are usually encountered. The results obtained in the Sri Lankan experiments are not comparable to those in the Philippines where the conditions are different.

No detailed examination of fish for symptoms of diseases was attempted because no large-scale mortality was reported. It would appear from Table 4 that predatory action was the main cause for the fish mortality in the pens. Apart from predation by fish, reptiles, birds and mammals move into an area where a fish farm has been established (Ranson and Beveridge 1983). The fishpen tends to attract birds not only because of large numbers of readily detected fish but also because it provides a site to perch. The survival of *Chanos* during different months has not been ascertained but it could be assumed that the loss was greater during the early stages after stocking since the fingerlings are more susceptible to predation.

Measures to prevent predation such as complete cleaning of the pen prior to stocking and scaring birds and greater care may have a positive effect on the increasing survival.

The stomach contents of the milkfish in coastal waters consists of diatoms, higher algae and rhyzopods and gastropods (Schuster 1952). In hypersaline ponds, milkfish feed extensively on benthic mats composed of halophilic bacteria, blue-green algae, diatoms and fungi (Crear 1980). The positive response of milkfish to artificial diets has been recorded by Lai (1966) and Chen (1981). The specific growth rates in the present experiments showed no significant effects of the 20% protein feed. Whether herbivores like milkfish can thrive on food of animal origin without digestive problems is a matter worthy of investigation (Lin 1969). Apart from the biochemical factors of the feed, the other reason that contributed to the nonsignificant effect of the feed was its form. Powdered rice bran and fishmeal tended to be carried out of the pen by the wind and wave action when released into the pen. Therefore, the period the feed is retained within the pen was inadequate to allow for effective consumption by the fish. Gut analyses, however, have not been carried out to confirm this.

In this experiment fish which were fed showed a slight decrease in the growth rate after 60 days, i.e., during February and March and showed acceleration of growth from May onwards. Fish which were not fed also followed more or less the same pattern. Salinity dropped to 24 ppt in March and gradually rose up to 40 ppt in July. This salinity fluctuation registered no impact on the growth of the fish.

Tampi (1960) obtained the values of $L_\infty = 31.5$ cm and K = 2.31 year for *Chanos chanos* from pond culture in India. The L_∞ value obtained here (Table 3) is higher and the K value is lower than that obtained by Tampi. However, these values cannot be compared because the results are obtained under different environmental conditions.

Acknowledgements

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Table 1. Experimental conditions of the pen culture.

Experimental conditions	Pen no. 1	Pen no. 2
Species stocked	*Chanos chanos*	*Chanos chanos*
Number stocked	2,500	2,500
Ave. length at stocking (cm)	10.9	10.6
Ave. weight at stocking (g)	11.6	10.0
Date of stocking	20 December	20 December
Feed used	Rice bran : fish meal 8 : 2	–
Rate of feeding	3% body wt. daily	–
Date of harvest	24 June	3 July
No. of days of culture	190	200

Table 2. Growth of fish recorded

	Pen no. 1	Pen no. 2
Ave. length at harvest (cm)	27.8	26.6
Ave. weight at harvest (g)	130	118.5
Growth rate (g/day)	0.62	0.54
Total no. harvested	1,654	1,603
Total weight at harvest (kg)	215	190
Survival (%)	66.2	64.12
Instantaneous rate of mortality (Z)/year	0.794	0.81

Table 3. Calculated Bertalanffy parameters.

Pen no.	L_∞ (cm)	K (yr^{-1})
Pen no. 1	47.89	0.925
Pen no. 2	50.61	0.807

Table 4. Fish species observed in the pens during the culture period.

Opisthopterus tardoore
Leiognathus sp.
Psettodes erumei
Sillago sihama
Etroplus suratensis
Ephippus orbis
Otolithus sp.
Glossogobius sp.
Sphyraena sp.
Lutjanus sp.
Triacanthus sp.
Elops sp.
Arothron stellatus
Mugil cephalus
Macrura kelee
Stolephorus indicus
Ablennes sp.
Scatophagus argus
Allanetta sp.
Osteogeneiousus militaris
Carangoides sp.
Chorinemus sp.
Acanthopagrus latus
Epinephelus sp.
Johnius sp.

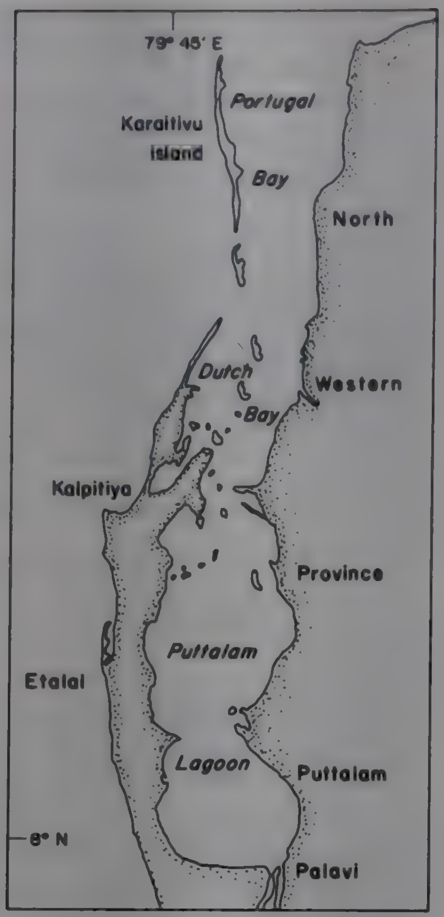
79° 45' E
Portugal
Karaitivu
island
Bay
North
Dutch
Bay
Western
Kalpitiya
Province
Puttalam
Etalai
Lagoon
Puttalam
8° N
Palavi

Fig. 1. Location of Puttalam Lagoon.

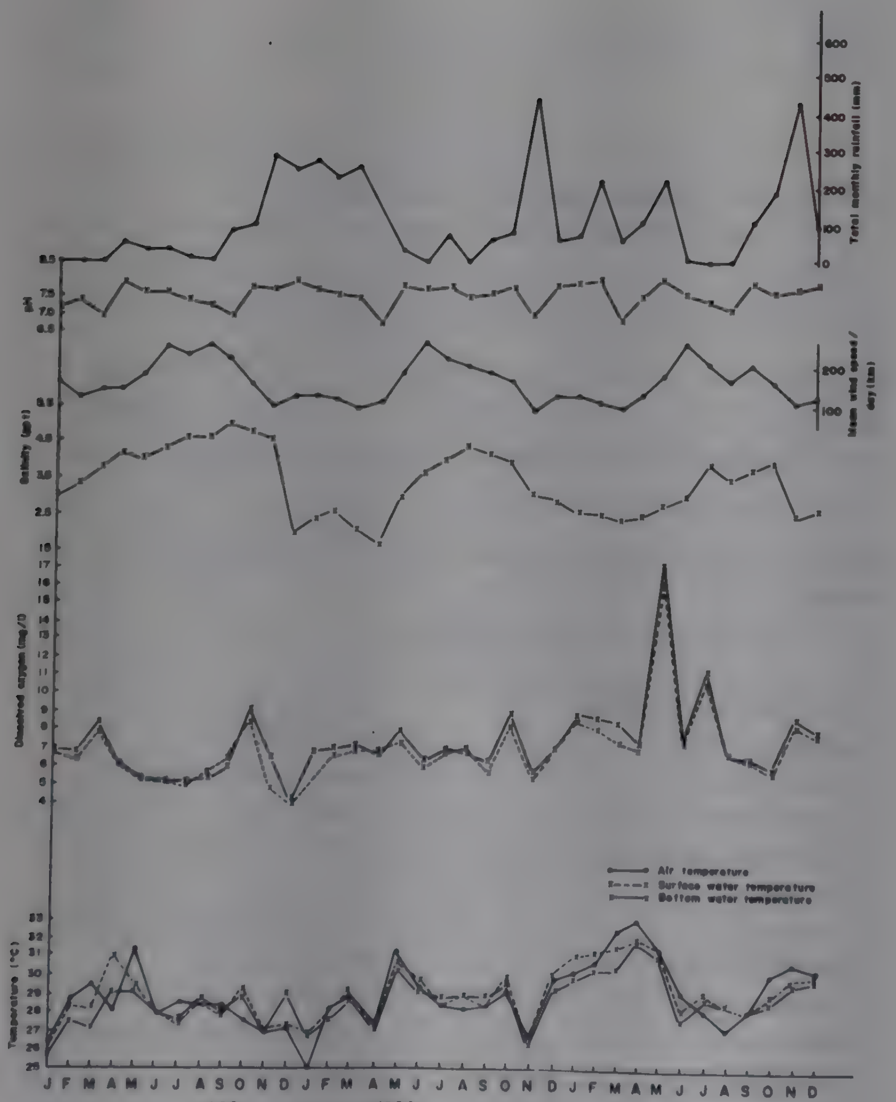

Fig. 2. Some hydrological conditions at the experimental culture site.

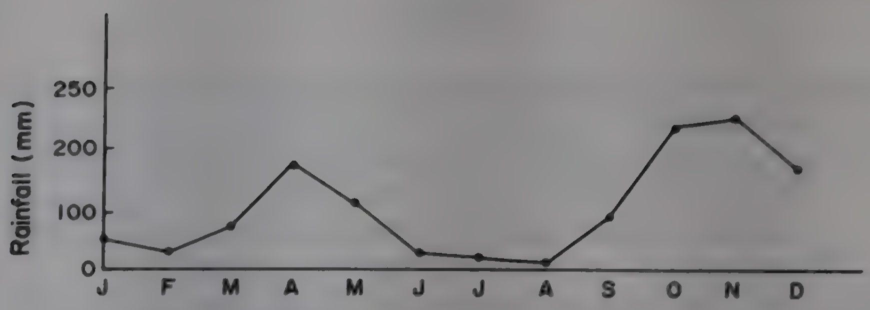

Fig. 3. 30-year average monthly rainfall (mm) 1951-1980.

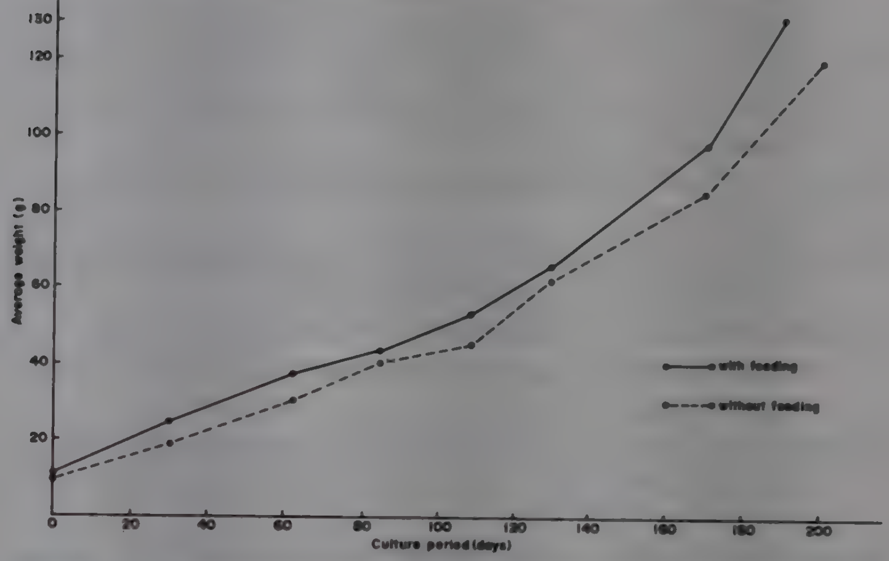

Fig. 4. Growth curve for milkfish from fingerlings to 200 days culture in experimental pens.

Intensification Techniques in the Extensive Culture of *Penaeus monodon*

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Abstract

Traditional shrimp *(Penaeus monodon)* farming is characterized by low yields, 100-300 kg/ha/yr. To provide farmers with appropriate technology that can immediately improve pond yield with minimal investment, experiments were conducted in brackishwater earthen ponds in two phases. The first phase was to evaluate the optimum stocking density without transfer of stock during the culture period up to harvest time. In the second phase, the best stocking density in the first phase was selected to determine the effect of transfer to newly-prepared ponds at 45-day and 60-day intervals. In the first phase, the ponds were stocked with hatchery-bred fry at the stocking rates of 1, 2 and 4/m^2 with no supplementary feeding for the first two months of culture. Feed was introduced after the second month at 6% body weight for the third month and at 4% body weight on the fourth. The yields after four months were 171 kg., 317 kg and 382 kg/ha/crop with survival rates of 85%, 83% and 74%, respectively. The stocking density of 2/m^2 was selected for the second phase experiment. The yield was 422 kg, 525 kg and 372 kg/ha/crop at 45 days and 60 days transfer and nontransferred, respectively.

Introduction

The present trend towards intensification of stocking density in tiger shrimp farming is increasing. Intensification differs from the traditional extensive method in terms of culture operation, yield and capital investment (Shigueno 1975; Liao 1977). About 80% of the shrimp ponds in Southeast Asia still use traditional, extensive culture methods. Yields are low (100 to 300 kg/ha) and less profitable than in intensive culture.

This study was aimed at increasing production through intensification in traditional culture operations without substantial increase in scale or initial investment. The assumption is that the natural food in a fertilized pond can support densities of shrimp larvae higher than the traditional stocking rate of 5,000/ha within the first two months after stocking. Artificial feeds (formulated or fresh) were then applied as supplement.

Materials and Methods

The experiments were conducted in two phases. The first phase was to evaluate the optimum stocking density without transfer of stock during the culture period up to harvest time. In the second phase, the appropriate stocking density was selected to evaluate the effect of stock transfers on the growth and survival rate of shrimp.

Nine 300-m^2 brackishwater ponds with average depth of 1.0 m. were used. Each pond had both inlet and outlet made of PVC standpipes 15-cm dia. Single net enclosures (0.5 mm mesh) were installed inside the ponds in front of the inlet as well as the outlet pipes to keep out predators and other undesirable organisms as well as to prevent the escape of the cultured shrimps.

Two weeks before stocking, the ponds were dried until the bottom cracked. After drying, Dolomitic lime ($CaMgCO_3$) was applied to the dikes as well as the pond bottom at 3 t/ha. Chicken manure was applied at 600 kg/ha. Water was then admitted into the ponds to an average depth of about 20-30 cm. Inorganic fertilizer was then broadcast at 37.5 kg/ha of ammonium phosphate (16-20-0) and 12.5 kg/ha of urea (46-0-0). The ponds were left undisturbed during the growth of the natural food. Fresh seawater was then allowed into the pond at 80-100 cm.

Depth of water inside the ponds was maintained at 80-100 cm throughout the experiment period. Changing of pond water was solely dependent on tidal fluctuation; thus, daily water management was effected only during the spring tides.

Dissolved oxygen, pH and salinity of the pond water were measured *in situ* twice daily at 0600 and 1400 hr.

A scheme of maintaining pond water productivity was initiated with the use of organic fertilizer. To each pond after the last change of water every spring tide, 25 kg of chicken manure wrapped in polypropylene bags were hung immediately opposite the water intake pipe.

The supplemental feed was a locally-formulated diet with a local ingredient (protein content 35%, moisture 10%) given twice a day at alternate interval. Feeding was done around 0700 and 0500 hr. Estimated feed required for the first supplementary feeding in the third month was 6% of total biomass and 4% in the fourth month.

At initial stocking the hatchery-bred postlarvae (P_{22}-P_{25}) had an average length of 1.5 mm and average weight of 0.09 g. The postlarvae were stocked in the early morning (0630-0700 hr). The stock was sampled a month after stocking and thereafter biweekly until harvest.

In phase one, shrimp were stocked at 1, 2 and $4/m^2$ with three replications. In phase two, the appropriate stocking density in the first phase ($2/m^2$) was selected. In this phase, there were three treatments. In the first treatment, the stock was transferred to a newly-prepared pond every 45 days of culture while in the second treatment, the stock was transferred after 60 days of culture. The third treatment served as control without any transfer of the shrimp.

Results

The entire grow-out period was 120 days. Average daily weight increments at stocking density of 1, 2, and $4/m^2$ were 0.16, 0.15 and 0.11 g, respectively (Table 1).

The yields showed significant differences ($p < 0.05$) between stocking levels at $1/m^2$ and $2/m^2$ (Table 2). However, the difference in size was not significant at both levels of stocking as both reached marketable size (20 to 30 g) in 120 days. Results also indicated no significant difference in yield between stocking levels at 2 and $4/m^2$ but they differed significantly ($p < 0.05$) in size at harvest. No significant differences were found among the survival rates. Size frequency distributions of harvested shrimp are illustrated in Fig. 1.

Overall yield at $1/m^2$ averaged 171 kg/ha/crop; at $2/m^2$ yield averaged 317 kg/ha/crop and at $4/m^2$, 383 kg/ha/crop.

The growth rates of *P. monodon* transferred to newly prepared ponds were significantly different ($p < 0.05$) from that of the control (Table 3). Daily growth rates of 0.26 g and 0.27 g, respectively, were obtained from transferred animals while only 0.20 g was recorded from the control. Fig. 2 shows the overall growth patterns.

There were no significant differences in yield among the three treatments. The highest yield (525 kg/ha/crop) shown in Table 4, was derived from treatment B where the shrimp were transferred 60 days after stocking. From those transferred every 45-day intervals the yield was only 422 kg/ha/crop. Sizes of transferred shrimp were significantly different ($p < 0.05$) from those not transferred (Table 4).

The survival rate of 67.1% at 45-day transfers was the lowest compared to 80.8% and 74.5% at 60-day transfer and nontransferred, respectively.

Discussion

Results indicate that production from traditional shrimp ponds can be substantially increased with minimal input and capital.

At harvest the estimated mean weight of 31.6 and 32.6 g when transferred to new ponds at $2/m^2$ are similar to those cultured in an intensive system (Liao 1977; Kungvankij et al. 1976). However, the stocking rates in these experiments were much lower than in the intensive system.

The results confirm the major role of natural food in the growth of *P. monodon*. The abundance of food organisms promoted the rapid growth of the transferred animals.

The experiments also indicate that transferring shrimp may induce stress and cause high mortality due to handling. This was evidenced when the shrimp were transferred at 45-day intervals. Further studies are needed to reduce mortality. The results further demonstrate that the modular farm system can be applied to shrimp farming. With good farm management the farm can produce 5-6 crops/ha/yr.

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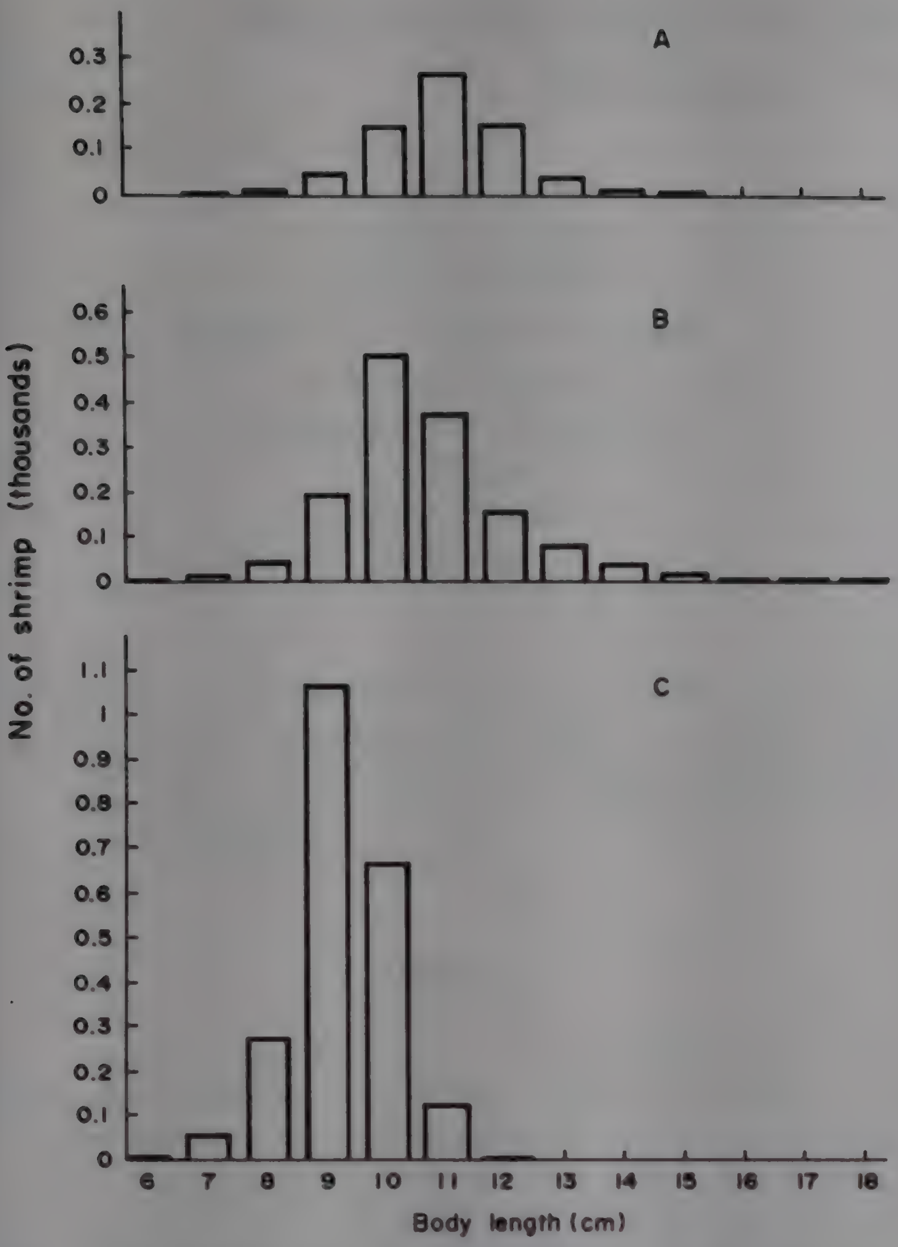

Fig. 1. Size distribution of harvested *P. monodon* stocked at $1/m^2$ (A); $2/m^2$ (B) and $4/m^2$ (C).

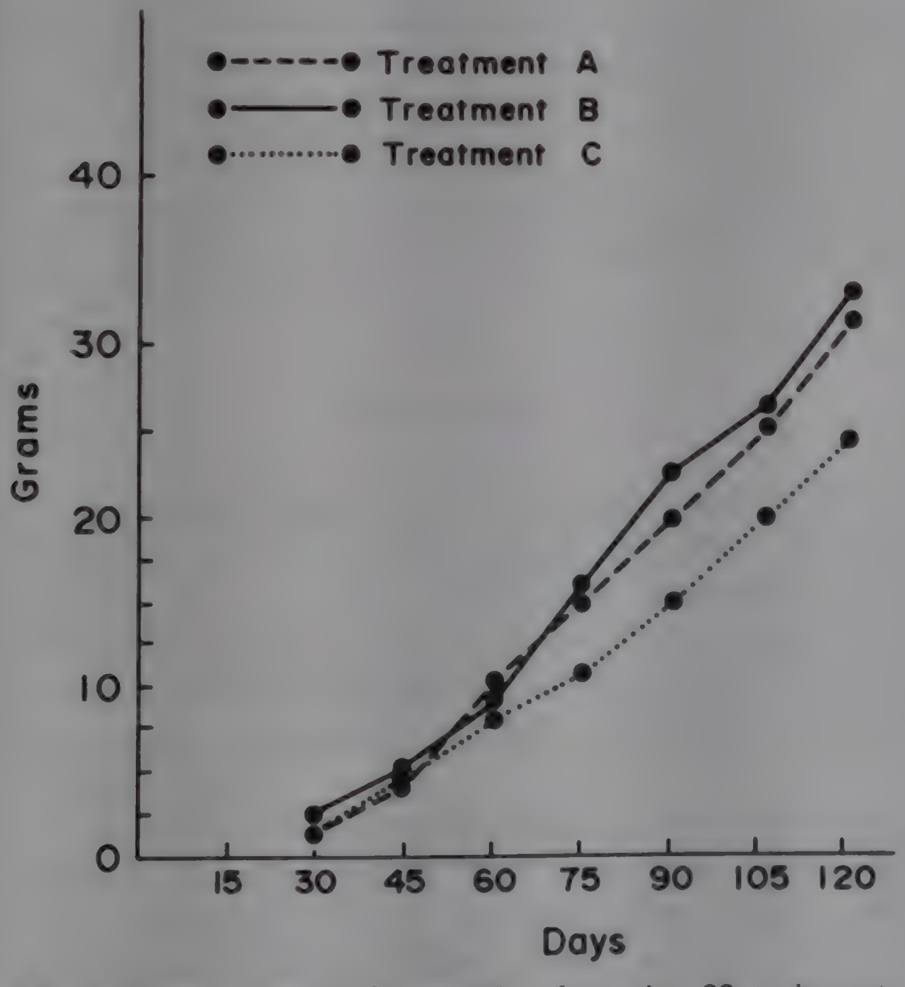

Fig. 2. Growth rates of *P. monodon* from day 30 to harvest, transferred at 45 days (Treatment A); 60 days (Treatment B); and not transferred (Treatment C).

Table 1. Average daily weight increment and size at harvest (Phase I).

Stocking density (no./m^2)	Initial weight (g)	Weight at harvest (g)	Daily increase (g)
1	0.09	20.4±6.8	0.16
2	0.09	18.9±5.9	0.15
4	0.09	12.9±3.4	0.11

Table 2. Average body weight, survival rate and net production at various stocking densities (Phase I).

Stocking density (no./m^2)	Total harvest (kg)	Survival (%)	Average body weight (g)	Average net production (kg/ha/crop)
1	6.2	83.7	24.70	171
	4.8	79.0	20.25	
	4.4	91.0	16.16	
Average	(5.1^a)	(84.5^a)	(20.4^a)	
2	9.8	85.0	19.22	317
	7.8	74.0	17.48	
	10.8	90.0	20.02	
Average	(9.5^b)	(83.0^a)	(18.9^a)	
4	10.1	62.0	13.50	384
	14.8	78.0	15.80	
	9.3	81.0	9.60	
Average	(11.5^b)	(73.6^a)	(12.9^b)	

Note: Means followed by the same superscript are not significantly different at $p > 0.05$.

Table 3. Daily average weight gains of *P. monodon* (Phase II), when transferred to new ponds at 45 days (A), 60 days (B) and not transferred (C).

Treatment	Average initial weight (g)	Weight at harvest (g)	Daily growth (g)
A	0.09	31.6±8.3	0.26
B	0.09	32.6±9.0	0.27
C	0.09	24.6±7.0	0.20

Table 4. Survival rate, average body weight and production of *P. monodon* per crop at $2/m^2$ (Phase II), and transfer at 45 days (A), 60 days (B) and not transferred (C).

Treatment	Total harvest (kg)	Survival (%)	Average body weight (g)	Average production (kg/ha/crop)
A	13.8	72.5	31.7	460
	12.3	61.7	33.4	410
	11.9	67.3	29.7	396
Average	(12.6^a)	(67.1^a)	(31.6^a)	422
B	15.0	70.8	35.2	500
	17.3	90.5	31.8	576
	14.9	81.0	30.8	499
Average	(15.7^a)	(80.8^a)	(32.6^a)	525
C	8.6	49.2	20.8	289
	9.1	67.5	22.6	303
	15.8	86.7	30.4	526
Average	(11.1^a)	(74.5^a)	(24.6^b)	372

Note: Means followed by same superscript are not significantly different at $p > 0.05$.

The Effect of Paddlewheel Aerators on Ammonia and Carbon Dioxide Removal in Intensive Pond Culture*

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Abstract

In intensive pond fish culture, good water quality is critical for fish growth and survival. Various water management techniques have been developed to maintain adequate levels of dissolved oxygen and to prevent the accumulation of ammonia and carbon dioxide to toxic levels. This study investigated the effect of paddlewheel aerators on the removal of ammonia and carbon dioxide and to ascertain its well-established effect of maintaining optimum dissolved oxygen levels in ponds sustaining a high biomass. A 500-m^2 earthen pond was stocked with *Oreochromis niloticus* averaging 170 g each to attain a total biomass of 3,000 kg/ha. Un-ionized ammonia and carbon dioxide levels were monitored every four hours for both aerated and unaerated conditions in the same pond. Each treatment was monitored over 24-hour cycles. Results indicate a significant effect of aeration on the diel pattern for carbon dioxide but none on ammonia. Carbon dioxide levels accumulated through the night and peaked between 4 and 8 a.m. at which time aeration significantly reduced it. Ammonia concentration was highest at 4 p.m. regardless of treatment.

Introduction

In intensive pond fish culture, good water quality is critical for fish survival and growth. The high biomass sustained in the pond, coupled with fertilization and feeding can result in the accumulation of toxic metabolites such as un-ionized ammonia and carbon dioxide excreted as metabolic end-products of fish, other organisms and microorganisms.

Ammonia exists in pond water in two forms: un-ionized (NH_3) and ionized (NH_4^+). Its toxic effects are mainly associated with the un-ionized form, the concentration of which is related to total ammonia content, water pH and temperature (Eddy 1982). Carbon dioxide (CO_2) dissolves in water to form carbonic acid (H_2CO_3) which dissociates into bicarbonate (HCO_3^-) and hydrogen (H^+) ions. The HCO_3^- in turn dissociates further to form carbonate ($CO_3^=$) ions and more H^+. Carbon dioxide is relatively more toxic than the other forms, and high concentration can result in a significant decrease in pond water pH. The relative proportions of NH_3 and NH_4^+ and of CO_2, H_2CO_3 HCO_3^- and $CO_3^=$ are dependent on pH and temperature.

Water surface agitation exposes a larger portion of the water to the atmosphere. When a concentration gradient exists across the air-water interface, mass transfer occurs down the gradient to bring the atmospheric and dissolved gas concentrations into equilibrium. Since both NH_3 and CO_2 are in dissolved gaseous forms in the pond, it is possible that water surface agitation will effect their volatilization when gaseous concentrations are higher than atmospheric concentrations.

The paddlewheel aerator has long been established as a very effective device for adding dissolved oxygen (DO) to oxygen-depleted pond water (Boyd and Tucker 1979). Originally conceived as an emergency aeration device for use only during periods of low DO levels, such as the early morning hours or after massive phytoplankton die-offs, many fish farmers now operate it continuously throughout the day to remove toxic gaseous elements and maintain optimum levels of DO.

The results are to form the basis for the development of an improved water-quality management strategy for intensive pond culture with the use of paddlewheel aerators.

Materials and Methods

The experiment was conducted in a 500-m^2 earthen pond with an average water depth of 0.75 m. *Oreochromis niloticus* averaging 170 g each, total biomass 3,000 kg/ha, were stocked and fed a commercial diet containing 35% protein thrice daily at a rate of 2% body weight.

An aerator with two 60-cm paddlewheels was installed in the northern corner of the pond. The paddlewheels, powered by a 0.5-hp electric motor, rotated at about 138 rpm. Leaks in the aerator float were effectively monitored by periodic measurements of the current being drawn by the motor. A draw of 2-3 amperes was considered optimum.

Water temperature, pH, salinity, water depth, dissolved oxygen, un-ionized ammonia and carbon dioxide concentrations were monitored every four hours in two 24-hour cycles each for the aerated and unaerated conditions. Parameters for the two treatments were measured and the effects of aeration monitored. Weather conditions and the response of the fish were also noted. Water samples for pH, NH_3 and CO_2 determinations were drawn near the surface and bottom at three points in the pond: eastern and western and center. Temperature and DO were measured in the laboratory with a Corning pH meter Model 10. Total ammonia-nitrogen and CO_2 (alkalinity) were determined according to the methods described by Strickland and Parsons (1972) and APHA (1975), respectively. Un-ionized NH_3 concentrations were calculated using data from Emerson et al. (1975). A pH meter was used to determine alkalinity at the equivalence point at pH 4.5 as no sharp color change was observed at the methyl orange endpoint. Alkalinity values were then converted to total carbon dioxide levels according to Strickland and Parsons (1972).

The data were subjected to an analysis of variance to determine whether significant changes occurred at different periods over a 24-hour cycle and to compare the effect of paddlewheel aeration on NH_3 and CO_2 levels.

Results

Results are based on data collected over four continuous days of observation. Water salinity ranged from 34-35 ppt. Pond water was rather turbid, the pond bottom not visible even on sunny days. Water temperature was 24.5-30.8°C under aerated conditions and 26.5-31.4°C under unaerated conditions. Table 1 compares water quality parameter values between aerated and unaerated pond conditions. Figs. 1-4 compare DO, pH, CO_2 and NH_3 levels between the two conditions.

Carbon dioxide concentrations were significantly affected ($P < 0.05$) by aeration. Carbon dioxide levels peaked at around 4 a.m. until aeration significantly reduced it. At this time, the mean CO_2 level under aerated conditions was 3.98 mmoles/l compared to 4.36 mmoles/l under unaerated conditions. At a mean 24-hour concentration of 2.83 mmoles/l in this high biomass pond under unaerated conditions, CO_2 levels were significantly higher than the average of 0.50 mmoles/l in an adjacent pond sustaining a biomass one magnitude lower.

There were no significant differences ($P > 0.05$) in NH_3 concentrations between an aerated and an unaerated pond. Ammonia and pH levels were highest at 4 p.m. and lowest at 4 a.m. regardless of treatment. The average total ammonia concentration of 0.18 mg/l, however, was significantly higher than the average of 0.08 mg/l in the adjacent low biomass pond.

The weather on all four days of observation was fair with moderate wind and sparse clouds. However, between 4 p.m. and 12 midnight on day 4 (unaerated condition), the wind stopped blowing. This sudden change in weather may have been the cause of the plunge of pH and DO levels to a critical low of 6.88 and 2.88 mg/l, respectively at 12 midnight. Some fish were observed to be at or near the water surface while a few were observed leaping out of the water.

Discussion

The results clearly demonstrate the significant increase in pond carbon dioxide and total ammonia levels and depletion of DO to limiting levels due to crowding of fish. Low pH, high CO_2, ammonia, nitrite and low oxygen are all deleterious to fish health, impair growth, and result in a variety of other sublethal effects (Randall et al. 1982). Un-ionized ammonia can readily diffuse across gill membranes with its lipid solubility and lack of charge (Randall et al. 1982) and sublethal concentrations may reduce growth, damage dorsal fins, pectoral fins and other organs, cause gill hyperplasia, thickening of the gill epithelieum, fusion of lamellae, swollen gills and hemorrhaging and predispose fish to disease (Colt and Tchobanoglous 1978; Redner and Stickney 1979; Randall et al. 1982; Soderberg et al. 1983). Hypercapnia, a condition resulting from an increase in ambient CO_2, typically causes a rapid decrease in blood pH due to elevated arterial partial pressure of CO_2 in fish (Randall et al. 1982).

Significant changes in diel pattern of pH, CO_2, NH_3 and DO were observed in the pond with high stocking density. The pattern follows that which results from photosynthesis of pond flora and respiration of the fish and other organisms. With sunlight, photosynthesis brings about an increase in DO to a late afternoon maximum and a reduction in the concentration of dissolved CO_2 to a late afternoon minimum. During the dark, continuing respiration results in diminishing DO and accumulation of CO_2 with their respective minimum and maximum occurring around dawn. Changes in pH correlated negatively with CO_2 concentrations, indicating that the buffering capacity of the pond saline water was not adequate to prevent pH fluctuations of 6.88-8.11. Changes in pH altered the relative concentration of un-ionized NH_3. At equilibrium, the ratio of NH_3 to total NH_3-NH_4^+ will increase as pH increases and decrease as pH decreases. The resulting peak NH_3 level at 4 p.m., corresponding with the peak water pH is consistent with this condition. At a particular pH, the concentration of ammonia also correlates positively with temperature since the equilibrium constant, K, for the system, increases with temperature.

The ranges of pH, DO and CO_2 concentrations were wider and the peaks more pronounced without aeration. These results indicate the positive effect of aerators on the maintenance of optimum DO levels and on the removal of CO_2 from the pond water. Maximum removal occurs at 8 a.m. when CO_2 levels are high, DO levels are low and photosynthesis is not yet significant. Removal of CO_2 by aeration at this time is particularly significant because the most detrimental effect of CO_2 in fish culture occurs at high CO_2 and low DO levels (Boyd 1979).

Ammonia was not significantly removed by the paddlewheel aerators even at its peak levels at 4 p.m. NH_3 loss rates due to volatilization increased with increasing wind speed, temperature and pH, with significant volatilization at pH 9 (Weiler 1979). In this study, the maximum pH observed was only 8.11. It is possible that volatilization was not observed, as the ionized and un-ionized forms of ammonia were near equilibrium and their relative levels were mainly determined by temperature and pH. Variations in total ammonia levels may have resulted mainly from respiration, photosynthesis and nitrification.

The sudden cessation of the wind between 4 p.m. and 12 midnight on day 4 (unaerated condition) caused CO_2 levels to rapidly accumulate by as much as 3.27 mmoles/l. Un-ionized NH_3 levels dropped by 0.015 mg/l due mainly to the rapid decline in pH. This isolated occurrence underscores the effect of water surface agitation on the removal of carbon dioxide and further supports the other data gathered during the rest of the experiment. Water quality parameter values returned to normal after wind speed increased at midnight.

It is thus recommended that paddlewheel aerators be operated not only during periods of low DO levels but also when CO_2 levels are expected to accumulate to toxic levels, such as in the early morning hours, after massive phytoplankton die-offs, or when air is still. In addition to aeration, regular partial change of the pond water (flushing) to reduce the levels of toxic substances is recommended, as aeration cannot adequately remove metabolic wastes.

Acknowledgements

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Table 1. Water quality parameter values in aerated and unaerated ponds at 34-35 ppt.

Parameter	Aerated (24.5-30.8°C)		Unaerated (26.5-31.4°C)	
Min DO mg/l	4.1	12 midnight	1.1	4 a.m.
Max DO mg/l	9.0	12 a.m.	14.7	4 p.m.
Min pH	7.24	4 a.m.	6.88	12 midnight
Max pH	8.11	4 p.m.	8.09	4 p.m.
Min NH_3 mg/l	0.001	4 a.m.	0.002	4 a.m.
Max NH_3 mg/l	0.023	4 p.m.	0.033	4 p.m.
Min CO_2 mmoles/l	0.63	4 p.m.	0.68	4 p.m.
Max CO_2 mmoles/l	5.43	4 a.m.	5.09	4 a.m.

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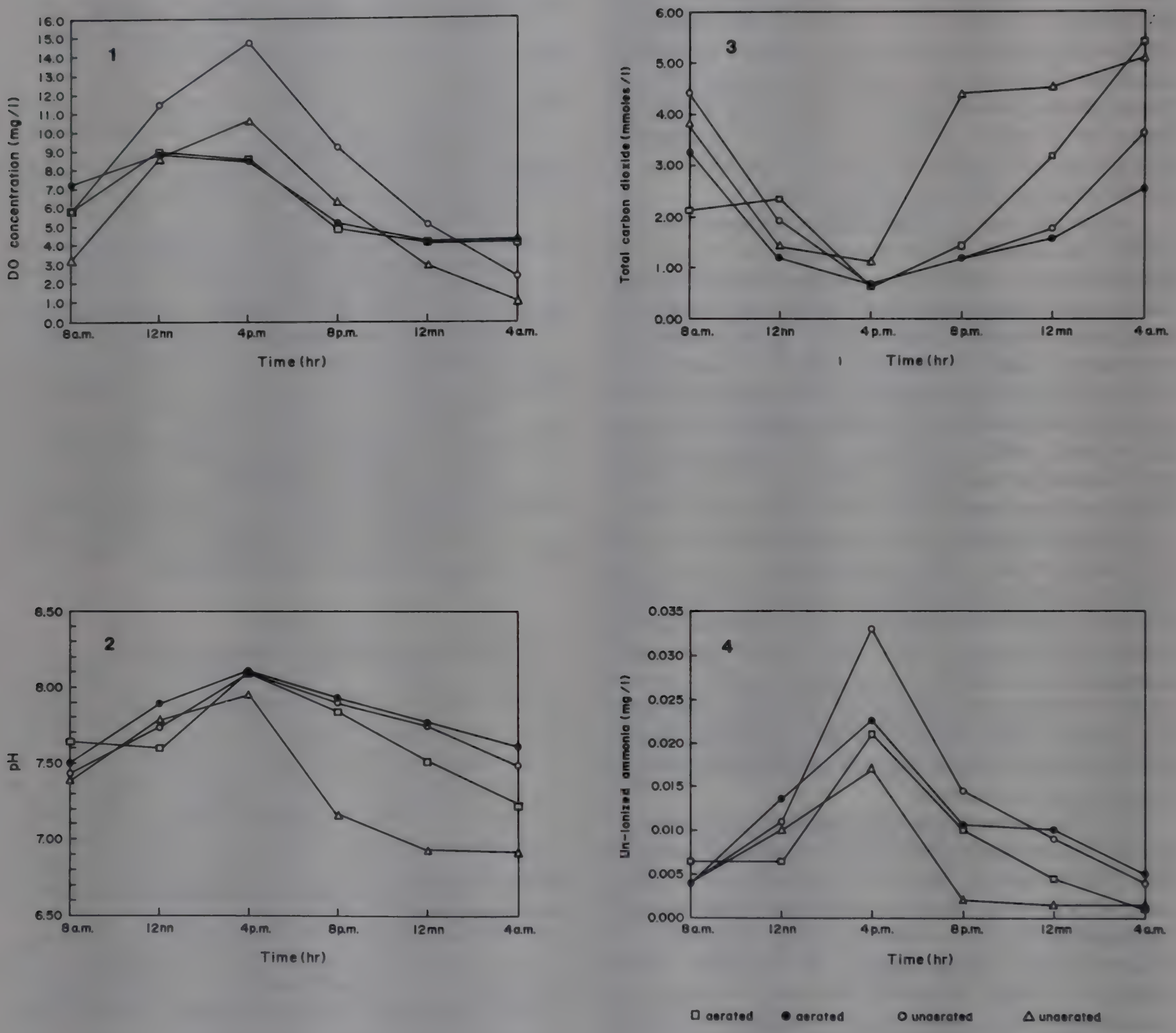

Figs. 1-4. Dissolved oxygen concentration, pH levels, and carbon dioxide and un-ionized ammonia concentrations, respectively, in the experimental pond over 20 hours. Two cycles for each parameter.

Triploidy Induced by Cold Shock in Cyprinid Loach, *Misgurnus anguillicaudatus*[1]

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Abstract

Optimum conditions for induction of triploidy by cold shock in the cyprinid loach, *Misgurnus anguillicaudatus*, were studied. Triploids were identified by measuring the DNA content of erythrocytes with microscope cytophotometry. When eggs were subjected to cold shock (8°C for 40 min.) at various times after fertilization, those shocked at 5 min. after fertilization produced the highest proportion of triploid fry. One hundred per cent triploids were produced in the groups treated at 1°C for 30-40 min. Hatching rate decreased and the proportion of deformed fry increased as cold shock duration was increased. The proportion of triploids tended to decrease as the temperature of cold shock increased from 1 to 12°C.

Introduction

Preliminary work on induction of polyploidy in aquatic animals was reported in the 1940s (for reviews, see Fankhauser 1945; Purdom 1983). It was not until the 1970s, however, that research on polyploid induction in finfish began in earnest (Purdom 1983). Subsequent technological development has been very rapid. Triploid induction is a favorable method of obtaining sterile strains to find a means of increasing the adult size and to avoid mortalities after spawning. Hence, the use of triploid induction could contribute to fish breeding and aquaculture (Donaldson and Hunter 1982).

Commercial seedling production of many freshwater and several marine fishes is practised in Taiwan. Recently, many trials aimed at improving propagation-related techniques to produce all-male populations and the availability of cryopreserved sperm have been undertaken but tests on the induction of triploidy have not been reported. Considering its high effectiveness and easy manipulation, cold shock (rather than heat shock and pressure shock) has been adopted as a means of inducing triploidy in loach by inhibiting extrusion of the second polar body during the second meiotic division of newly fertilized eggs. In loach, survival, growth and sterility of induced triploids were reported by Suzuki et al. (1985). This paper presents results of research to determine the optimal combination of cold shock conditions in cyprinid loach.

Materials and Methods

Female spawners were selected from captive broodstock and injected with bighead carp pituitary gland or HCG. Ripened eggs were stripped out of one gravid female. Testes were removed from two mature males and ground with 12 ml of Ringer's solution which contained 750 mg NaCl, 20 mg KCl and 40 mg $CaCl_2$ in each 100 ml H_2O. Sperm was activated by freshwater of three times volume and mixed with eggs immediately (arbitrarily termed the fertilization time). Thirty seconds after fertilization, the eggs were distributed into 60 petri dishes (6 replicates x 10 subexperimental groups) each containing 10 ml of freshwater. The number of eggs in each subexperimental group ranged from 350 to 500. Healthy eggs adhered to the bottom of the dish.

The experimental treatments and conditions are summarized in Table 1. In TRI-1, loach eggs were exposed to 8°C for 40 min. and the starting time of cold shock varied from 1 to 9 min. after fertilization in nine subexperimental groups. In Experiments TRI-2 to TRI-6, starting time and duration of cold shock were the same. However, the temperature of cold shock varied for Experiments TRI-2 to TRI-6. There were six replicates in each of the nine subexperimental groups and in the control. After cold shock treatment, eggs in petri dishes were rewarmed for 5 min. using water at room temperature. The numbers of eggs in each of a series of 60 petri dishes were counted.

Hatching rates in each petri dish were calculated after hatching. Twenty-four hours later, percentages of deformed fry in each petri dish and treatment were also recorded. The criteria for recognition of deformed fry were a curved tail and shortened body (Chen et al. 1986). Fry were reared for 20 days at a density not more than 80 individuals per container (11 x 18 x 5 cm) and fed with rotifera and brine shrimps.

Blood smears of 20-day old fry were prepared as described by Gervai et al. (1980) and Chen et al. (1986). DNA content of individual erythrocyte nuclei of five cells from 20 or fewer fish in each subexperimental group was measured and computed with a LEITZ MPV 3 microscope photometer. Each nuclei was read 32 times automatically.

Cytophotometric DNA assay was based on the quantity of light absorbed by the stained nuclei and was expressed as the absorption unit (in the absence of a flow cytometer). The readings of stains normally vary with (1) DNA content in the erythrocyte nuclei and (2) variation between and within each batch of stained slides. To evaluate the DNA content, the following equations were used:

$$CF = (F/Q) \times 6 \quad \text{and} \quad CFS = FS \times (CF/F)$$

where F is mean value of readings of each sampled fry; Q is mean value of readings of corresponding quantitative blank smear on the same slide (the blank was an erythrocyte smear from an untreated diploid loach); FS is standard error of readings of each fry; CF is corrected F; and CFS is corrected FS.

The difference between CF of each sample fry and the mean CF of the control group was determined by using the student's t-distribution. Differences were considered insignificant when $P > 0.01$. Since the control groups produced only diploids, triploidy was acknowledged when the CF on a slide of sample fry was 1.5 times that of the mean CF of the control group. In addition, individual mean erythrocyte DNA content expressed as absorption units of 100 erythrocytes from one assumed diploid and one assumed triploid group, respectively, was calculated and frequency histograms of DNA content were constructed.

Results

A frequency histogram of erythrocyte DNA content of one assumed diploid and triploid loach is shown in Fig. 1. No overlap was found. The mean erythrocyte DNA content of the assumed triploid was found to be 1.5 times that of the assumed diploid.

The hatching rate of eggs subjected to cold shock at 1-7 min. after fertilization was slightly lower than that of the control ($p < 0.02$; t-test). However, cold shock at 8-9 min. after fertilization produced no significant difference in hatching rate. The percentages of deformed fry in each experimental group were not significantly different from that in the control group (Fig. 2). The survival rates of 20-day old fry in the experimental groups ranged from 56.7% (8 min.) to 97.6% (4 min.) against the control (Table 2). Comparisons of the DNA content revealed that the highest percentage of triploids was found in the group started at 5 min. after fertilization. Earlier or later exposure resulted in lowered triploid rates. No triploids were found in the control group nor in groups started at 8 or 9 min.

The combined effect of temperature and duration of cold shock on the hatching rate and percentage of deformed fry is shown in Fig. 3. The hatching rate tended to decrease and percentage of deformed fry to increase as the duration of cold shock increased. Tables 3 and 4 show the survival of 20-day old fry. Exposure of eggs to 1°C for 20, 30, 40, 50 and 60 min.; 6°C for 30 and 40 min.; 8°C for 40 min. or 12°C for 40, 50, 60, 70 and 80 min. yielded over 80% triploid fish.

Discussion

The result from this study is in agreement with Suzuki et al. (1985) that cold shock of 1°C is a useful means of inducing triploidy in the cyprid loach. In this study, 1°C cold shock duration of 30 or 40 min. both produced 100% triploidy, better than that obtained by Suzuki et al. (1985) at 60 min. (84%) or those of our own results obtained at 20, 50 or 60 min.

The results indicate that triploidy increases as the cold shock temperature decreases. For those investigating temperatures between 0 and 1°C, cold shock will be of interest. Similarly, more data on the combined effects of cold shock temperature and duration will help to further elucidate the effects on the induction of triploidy in the loach.

Induction of triploidy is considered an effective method of improving growth in animals; however, attention should be drawn to other characteristics of some fish. For example, triploids of *Gasterosteus aculeatus* use less oxygen than diploids and their rate of respiration per unit area of body surface is reduced (Swarup 1959). A recent study of Atlantic salmon triploidy by Graham et al. (1985) found that a reduced ability to transport oxygen due to less hemoglobin-oxygen loading ratio for the triploids may hinder the triploids' ability to obtain oxygen under stressful conditions. Although triploid loach in this study appeared as viable as the diploids and no difficulties were experienced in raising them over the twelve-month duration of these experiments, the potential of using triploids to improve the culture of loach remains to be studied.

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Table 1. Summary of the experimental condition and treatment

Experiment	Temperature (°C)	Cold shock Timing (min. a.f.)[1]	Duration (min.)	Ambient temperature (°C)
TRI-1	8	1-9	40	29.7
TRI-2	12	5	5-80	28.5
TRI-3	8	5	5-80	28.0
TRI-4	6	5	5-80	24.0
TRI-5	4	5	5-80	27.2
TRI-6	1	5	5-80	27.2

[1] Min. after fertilization

Table 2. Survival rate and percentage of triploids in groups of 20-day old fish of eggs subjected to cold shocks (40 min. at 8 C) applied at various time intervals after fertilization; eggs in control runs given no cold shock. Experiment TRI-1

Start of cold shock (min. a.f.)*	No eggs	Fry surviving (no.)	Survival rate %	Survival rate % of control	Rearing for 20 days: No. of fish sampled	Diploid No	Diploid %	Triploid No	Triploid %
Control	70	50	71.4	100.0	20	20	100	0	0
1	150	90	60.0	84.0	20	18	90	2	10
2	175	90	51.4	72.0	20	17	85	3	15
3	145	96	66.2	92.7	20	17	85	3	15
4	155	108	69.7	97.6	18	16	89	2	11
5	160	96	60.0	84.0	20	12	60	8	40
6	150	88	58.7	82.2	20	16	80	4	20
7	170	116	68.2	95.5	20	19	95	1	5
8	195	79	40.5	56.7	19	19	100	0	0
9	230	153	66.5	93.1	20	20	100	0	0

*Min. a.f. = min. after fertilization

Table 3. Survival rate of 20-day old loach from eggs subjected to cold shock of various duration starting at 5 min. after fertilization

Temp. °C	12		8		6		4		1	
Duration (min.)	a*	b*	a	b	a	b	a	b	a	b
0	80.2	100	92.0	100	88.6	100	51.9	100	77.1	100
5	53.8	67.1	80.7	88.5	70.0	79.0	44.4	85.5	76.4	99.1
10	53.7	67.0	82.8	90.0	61.3	69.2	39.1	75.3	76.8	99.6
20	36.4	45.4	37.0	40.2	59.3	66.9	41.5	80.0	77.6	100.6
30	47.3	59.0	82.9	90.0	11.8	13.3	37.6	72.5	72.8	94.4
40	52.7	65.8	72.8	79.1	20.0	22.6	12.9	24.9	60.6	78.6
50	32.9	41.4	30.1	32.7	9.6	10.8	33.3	64.2	54.3	70.4
60	50.0	62.4	27.1	29.4	42.5	48.0	0	0	63.0	81.7
70	36.6	45.7	18.1	19.7	50.0	56.5	–	–	75.0	97.2
80	19.1	23.8	33.3	36.2	–	–	–	–	–	–

*a: survival rate (%)
b: survival rate against control (%)

Table 4. Percentage of triploid 20-day old loach from eggs subjected to cold shock of various duration starting at 5 min. after fertilization (Exp. TRI-1 to TRI-6).

Temp. °C	12		8		6		4		1	
Duration (min.)	a*	b*	a	b	a	b	a	b	a	b
0	10	0	20	0	16	0	20	0	20	0
5	8	6	20	0	16	0	20	0	20	5
10	16	38	20	35	15	20	20	15	20	15
20	19	68	20	75	14	71	20	30	20	80
30	20	85	20	70	8	100	20	60	20	100
40	17	94	20	80	10	80	4	75	20	100
50	20	90	19	68	6	67	1	100	15	86
60	19	84	20	60	15	73	–	–	5	80
70	19	89	20	70	13	92	–	–	5	20
80	20	80	20	40	–	–	–	–	–	–

*a: number of fish sampled
b: percentage of triploidy (%)

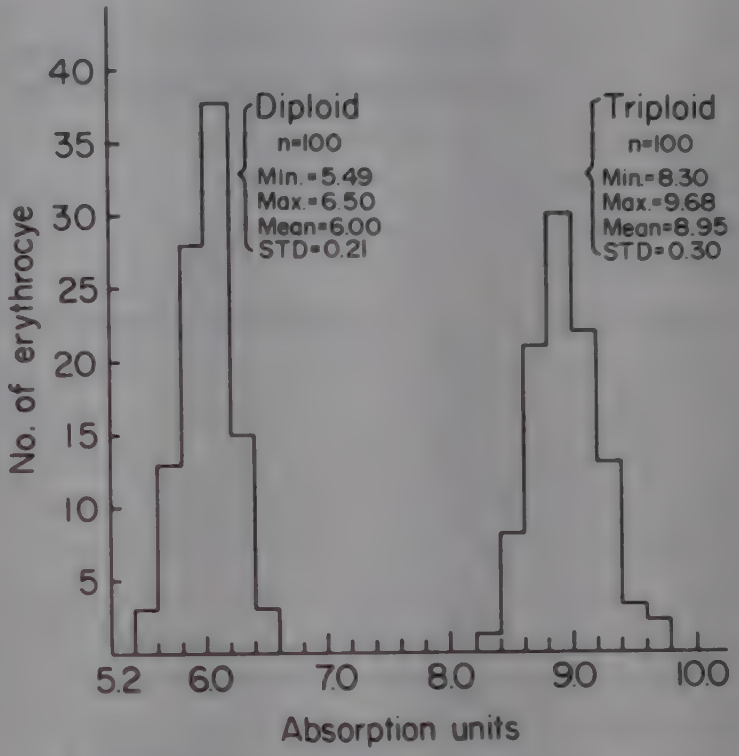

Fig. 1. Frequency histogram of erythrocyte DNA content in one diploid and one triploid loach. The readings of absorption units were determined by using microscope photometry.

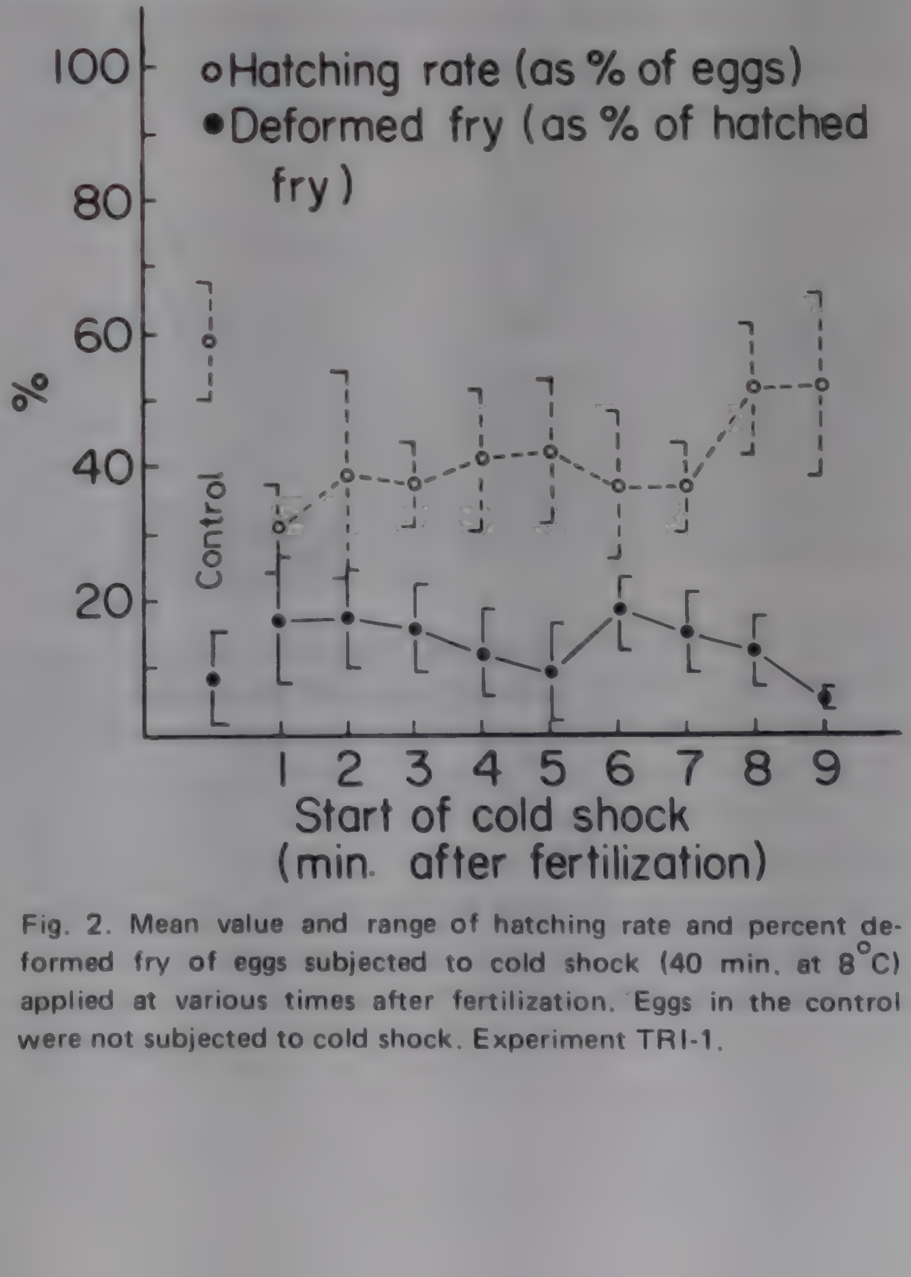

Fig. 2. Mean value and range of hatching rate and percent deformed fry of eggs subjected to cold shock (40 min. at 8°C) applied at various times after fertilization. Eggs in the control were not subjected to cold shock. Experiment TRI-1.

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[1] Contribution A No. 64 from the Tungkang Marine Laboratory.

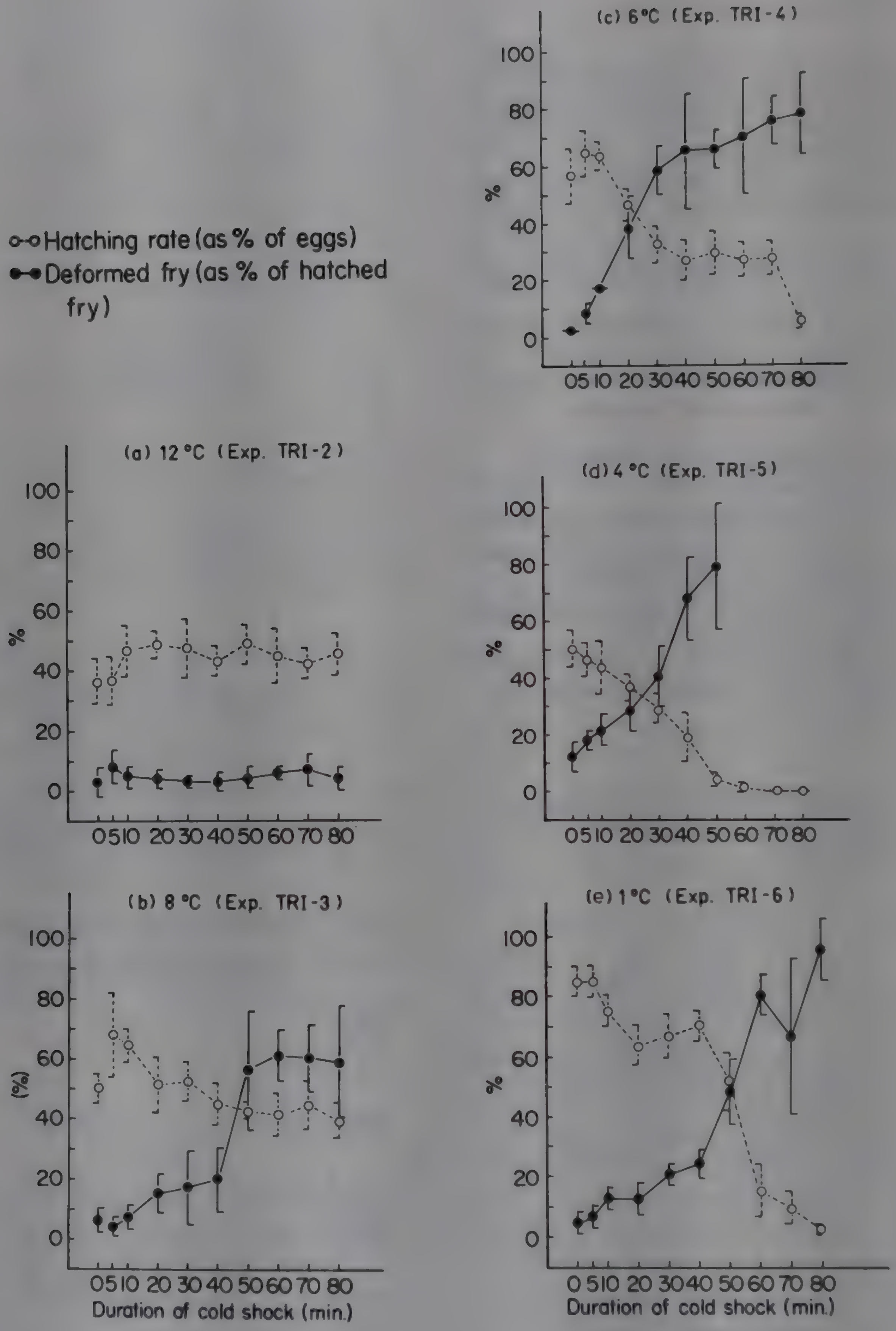

Fig. 3. Mean value and range for hatching rate and percentage of deformed fry from eggs subjected to cold shocks of various temperatures and duration started at 5 min. after fertilization.

Diploid Gynogenesis Induced by Cold Shock in Cyprinid Loach, *Misgurnus anguillicaudatus*[1]

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Abstract

A reliable method for the induction of heterozygous diploid gynogenetic fry in the cyprinid loach, *Misgurnus anguillicaudatus*, was developed by means of cold shock treatment to eggs fertilized with UV-irradiated sperm. Haploid and diploid fry were identified by measuring the DNA content of erythrocytes with the use of cytophotometry. Haploid fry were totally deformed and died within four days after hatching. All morphologically normal fry were confirmed to be diploid. UV-irradiation dosage of 0.5 to 2 J/cm^2 was the optimum for genetic inactivation of sperm of cyprinid loach. Eggs fertilized with UV-irradiated sperm and subjected to cold shock of 8°C for 40 min., at 3 to 5 min. after fertilization, resulted in comparatively high percentages of normal fry. As for the optimal cold shock temperature and duration for the treatments applied 4 min. after fertilization, high hatching rate and high rate of normal fry were produced from cold shock of 8°C for 40-60 min. and 6°C for 20-50 min.

Introduction

Gynogenesis is one type of parthenogensis through which embryos are obtained from the eggs without genetic contribution from the sperm. Natural gynogenesis has been recorded in species of the family Poeciliidae and the species *Carassius aureatus gibelio*. Since artificial diploid gynogenesis was first induced in frogs by Hertwig in 1911, the technique has been used in several commercial fish species and many reports on it have been published (for reviews see Purdom 1983; Chourrout 1984).

The main interest in inducing diploid gynogenesis lies in the production of inbred lines and monosex populations. Purdom (1983) estimated that one generation of gynogenesis is equivalent to about seven generations of full sib mating. Artificial gynogenesis in species having female homogamety will produce all female fry and the species having female heterogamety (ZW) will produce 1:1 of ZZ male and WW female. The WW super females can be crossed with normal ZZ males to produce all-female offspring (ZW). Artificial gynogenesis can also be used to study sex determination, gene-mapping, isolating mutants and preparing karyotypes (Gervai et al. 1980).

Production of diploid gynogenetic individuals requires the combination of sperm inactivation and diploidization of the maternal chromosome set. Gamma, X and UV irradiation can be used to inactivate sperm. UV light is inexpensive, can easily be set up in individual laboratories and is safer than gamma irradiation or Xrays (Lou and Purdom 1984). Cold shock, heat shock, hydrostatic pressure and various chemicals have been used for inducing gynogenesis and polyploidy in fish (Chourrout 1984). Suzuki et al. (1985) have reported work on survival, growth and fertility of gynogenetic diploids induced in the cyprinid loach, *Misgurnus anguillicaudatus*. However, data on the optimum condition for inducing diploid gynogenesis by applying cold shock to the eggs after fertilization with UV-irradiated sperm have not been reported.

The objective of this study was to determine the optimal condition for mass production of diploid gynogenetic cyprinid loach with combinations of UV-irradiation dosage, cold shock temperature, duration of treatment and length of time after fertilization.

Materials and Methods

The experiments were carried out at the Tungkang Marine Laboratory. Broodstocks were purchased from private loach farms and were stocked at a density not higher than 200 g/m^2 in a pond with a surface area of 6.25 m^2 and with 10 cm of soil at the bottom of the pond. They were fed twice daily with commercial loach pellet feed and water fleas.

Soft-belly female spawners more than 15 g were selected from captive broodstock. Ovulation was induced by hormone treatment. The females were injected with bighead carp pituitary gland or HCG based on the weight of the loach. The amount of hormone treatment was prepared according to Chen and Su (1980) and Suzuki (1983). In most cases, with a moderate water temperature of 28°C, the females responded well 8-10 hours after the injection of the hormone. The males were not treated with the hormone.

Two males were used in each experiment. Sperm were diluted with 12 ml of Ringer's solution which contained 7.5 g NaCl, 0.2 g KC1 and 0.4 g $CaCl_2$ per liter of distilled water. For sperm inactivation, one ml of Ringer's sperm solution was spread out in a petri dish. Each petri dish containing the sperm was exposed to one 15 W UV germicidal lamp (254 nm) at a distance of 2 cm (0.05 W/cm^2 measured at the surface of the solution) for the desired time. During irradiation treatment sperm was shaken at 100 times/min.

Ripened eggs were stripped out of one gravid female. Fertilization was carried out by mixing 3,500-5,000 eggs with 10 ml of Ringer's sperm solution and 30 ml of freshwater (arbitrarily termed the fertilization time). Thirty seconds after fertilization, the eggs were transferred to 60 petri dishes containing freshwater. The eggs adhered to the bottom of the petri dishes within 10 seconds.

For cold shock treatment, the fertilized eggs were immersed in cold water maintained at desired low temperatures by a low temperature water bath.

The experimental treatments and conditions are summarized in Table 1. Throughout the study, six replicates were used. In experiment GY-1, the effects of various UV-irradiation dosages on sperm inactivation were studied. In experiments GY-2 to GY-8, sperm were irradiated for a total dosage of 0.5 J/cm^2 over 10 seconds. In experiments GY-2 and GY-3, the cold shock temperature was fixed at 8°C and duration of cold shock at 40 min. to study the optimum time for the application of cold shock after fertilization. In experiments GY-4 and GY-8, the time of application of cold shock was fixed at 4 min. after fertilization to study the optimum cold shock temperature and duration.

After cold shock treatments, eggs in the petri dishes were transferred to water at room temperature for 5 min. The number of eggs in each petri dish was counted. The eggs in the petri dish were then incubated without aeration. Dead eggs were removed and water changed every six hours. Hatching rates were calculated in percentages of the total number of eggs in each petri dish. Normal and deformed fry in each petri dish were distinguished by their appearance at 24 hours after hatching. Deformed fry showed curved tail and short body.

Blood smears of 2-day old fry were made on slides and fixed in methanol-formalin 9:1 v/v for 15 min. Slides were washed twice with distilled water, dried and stained by the Feulgen reaction procedure (Humason 1979).

The DNA content of erythrocyte nuclei was measured (10 nuclei per fry) with LEITZ MPV 3 microscope photometer (Gervai et al. 1980; Johnstone 1985). Each nuclei was read 32 times automatically.

Results

Haploid gynogenesis by UV irradiation of sperm. In experiment GY-1, eggs fertilized with UV-irradiated sperm of dosage lower than 2 J/cm^2 showed slightly lower hatching rate than those in the control, but the difference was not significant. Hatching rate of the group at 3 J/cm^2 was significantly lower than that of the control ($P < 0.01$) (Fig. 1). All fry hatched from UV-irradiated sperm were deformed and died within four days after hatching. The effect of UV dosage on the erythrocyte DNA content of 2-day old fry in the samples is presented in Table 2. The average DNA content of erythrocyte decreased with increased UV-irradiation dosage. The difference of DNA content among UV-irradiated groups of 0.5 J/cm^2 or higher was found to be insignificant. Fry obtained from these groups were confirmed to be haploid. The gynogenetic haploid and diploid control fry are shown in Plate 1A & B.

Diploid gynogenesis by cold shock. Experiment GY-2 showed that nearly all fry were normal in control A (normal sperm; no cold shock). Control B (irradiated sperm; no cold shock) resulted in all deformed fry. More than 65% normal fry were obtained from treatments of 3 and 6 min. after ferilization. Treatments of 9 to 21 min. produced nearly all deformed fry (Fig. 2a).

Both the normal fry obtained from cold shock treatment and control A showed the normal morphological appearance (Plate 1B and C) and the same DNA content (Fig. 3). All normal fry from the treatment groups were confirmed to be gynogenetic diploids. However, some of the abnormal fry showed the same DNA content as untreated control A.

Based on the results of experiment GY-2, effects of cold shock started at 1-8 min. after fertilization were studied in experiment GY-3. More than 80% normal fry were obtained from 1 to 6 min. after fertilization and the best result was at 5 min. after fertilization. The differences among 3, 4 and 5 min., however, were not significant (Fig. 2b).

In experiments GY-4 to GY-8, the best results were obtained from a cold shock temperature of 8°C with duration of 40-60 min. and 6°C for 20-50 min. Cold shock of 12°C was not effective in producing gynogenetic diploid fry although the hatching rate was high. Cold shocks of 1 and 4°C resulted in significantly lower hatching rates than the control (Fig. 4).

Discussion

In our experiments on cyprinid loach, a reliable method of gynogenetic production was developed. UV irradiation has a marked effect on the genetic inactivation of sperm. Stanley and Jones (1976) used UV-irradiation dosage of 0.9 J/cm^2 for genetic inactivation of sperm in grass carp, *Ctenopharyngodon idella*. Suzuki et al. (1985) found that the UV-irradiation dosage of 0.12 J/cm^2 for sperm inactivation in cyprinid loach resulted in high fertilization rate. In our experiment GY-1, UV-irradiation dosage of 0.5-2 J/cm^2 was found to be the optimum UV-irradiation for genetic inactivation of sperm of the cyprinid loach. This difference may be the result of different UV illumination or stock of fish used.

This study shows that the optimum starting time for cold shock treatments was 3-5 min. after fertilization at an ambient temperature of 29°C. This result corresponded with that of Suzuki et al. (1985). Cold shock given 9 min. after fertilization failed to restore diploidy. This showed that the second polar body had already separated. Hatching rate, on the other hand, was found to be relatively independent of the time of application.

Suzuki et al. (1985) fixed the cold shock temperature at 1°C and duration of cold shock at 60 min. The effects of other temperature and duration on diploid gynogenesis were not reported in cyprinid loach. A low hatching rate was found in experiment GY-8 (cold shock at 1°C, 4 min. after ferilization). This result was not in agreement with that of Suzuki et al. (1985). Egg quality may be an important factor in this difference.

All normal fry resulting from cold shock treatment were confirmed to be diploids by their appearance and erythrocyte DNA content. Some abnormal fry from cold shock treatment had the same DNA content as fry of control A (normal sperm; no cold shock). These may be the result of homozygosity due to recessive genes.

Suzuki et al. (1985) have demonstrated the sex-determining gene of the cyprinid loach to be XX female homogametic type. Artificial gynogenesis can be used to produce all female offspring. However, this preliminary study showed that the survival rate was low in the diploid gynogenetic group of cyprinid loach. Further investigations on the mass production of all female fry are needed.

Acknowledgements

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Table 1. Summary of the experimental treatments.

Experiment	UV-irradiation dosage (J/cm^2)	Temperature (°C)	Cold shock Timing (min a.f.)*	Duration (min.)	Ambient temperature (°C)
GY-1	0.3	–	–	–	28.0
GY-2	0.5	8	3-21	40	29.0
GY-3	0.5	8	1-8	40	29.2
GY-4	0.5	12	4	0-70	29.0
GY-5	0.5	8	4	0-60	27.5
GY-6	0.5	6	4	0-70	27.8
GY-7	0.5	4	4	0-70	27.2
GY-8	0.5	1	4	0-70	26.0

*min a.f. = min. after fertilization.

Table 2. Effect of UV dosage on the erythrocyte DNA content of 2-day old loach fry (experiment GY-1).

UV irradiation dosages (J/cm^2)	Number of fry	Type of fry	Average DNA content (A.U.)*	S.E.
0	20	normal	5.83	±0.53
0.05	20	deformed	3.72	±0.31
0.1	20	deformed	3.74	±0.38
0.3	20	deformed	3.87	±0.61
0.5	20	deformed	3.55	±0.32
1	20	deformed	3.55	±0.26
1.5	20	deformed	3.52	±0.33
2	20	deformed	3.50	±0.32
3	20	deformed	3.50	±0.26

*A.U. = arbitrary units.

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[1] Contribution A, No. 63 from the Tungkang Marine Laboratory.

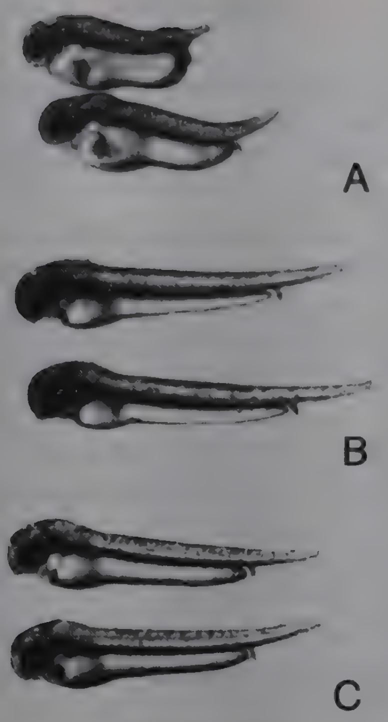

Plate 1. Two-day-old fry of gynogenetic haploids (A), control diploids (B) and gynogenetic diploids (C) of cyprinid loach, *Misgurnus anguillicaudatus.*

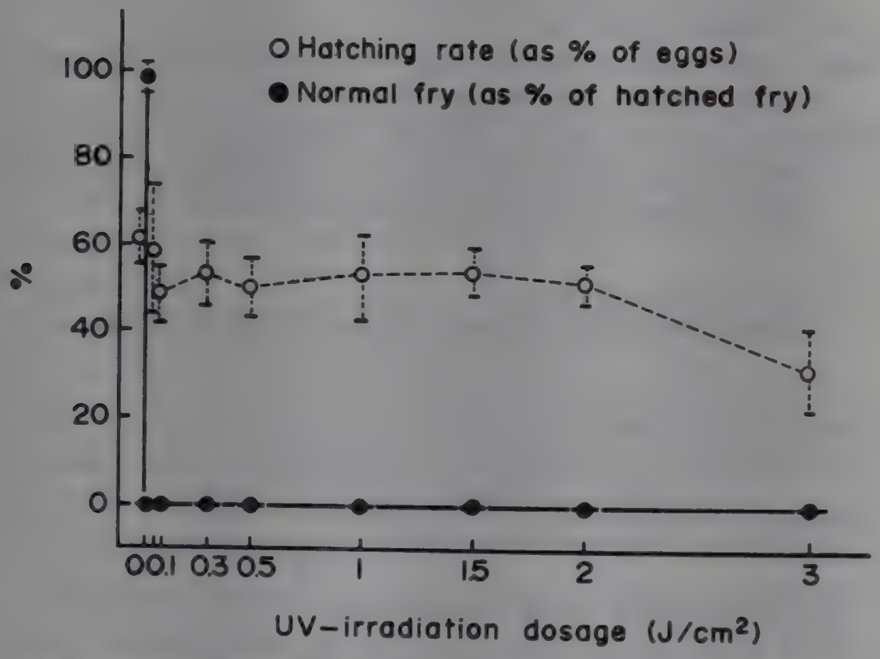

Fig. 1. Effect of UV dosage on hatching rate and the percentage of normal fry. Experiment GY-1.

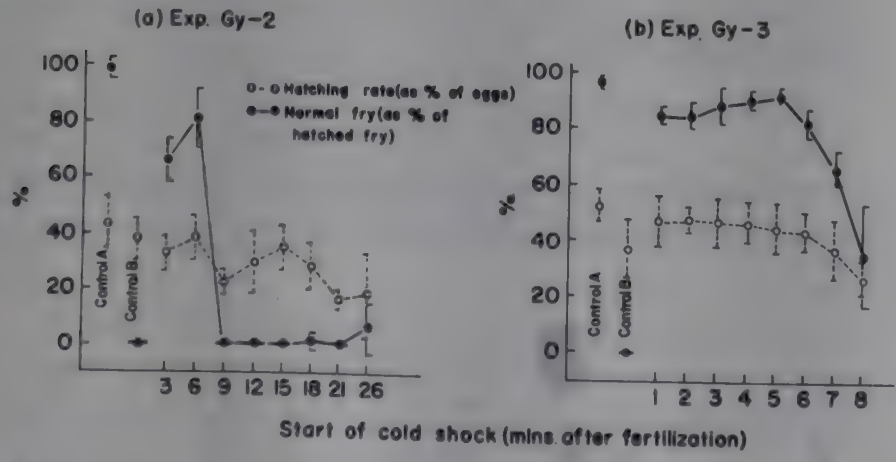

Fig. 2. Mean value and range for hatching rate and percent normal fry of eggs fertilized with UV-irradiated sperm and subjected to cold shock (8°C, 40 min.) at various time intervals after fertilization. Both controls comprised eggs not subjected to cold shock. Irradiated sperm used in all treatments except control A. ○—○ hatching rate (as % of eggs), ●—● normal fry (as % of hatched fry).

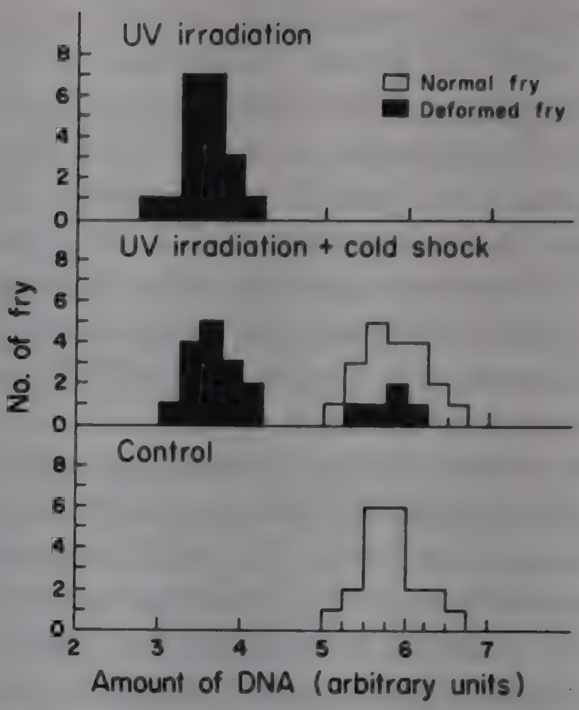

Fig. 3. Distribution of erythrocyte DNA content of 2-day-old fry in Experiment GY-2.

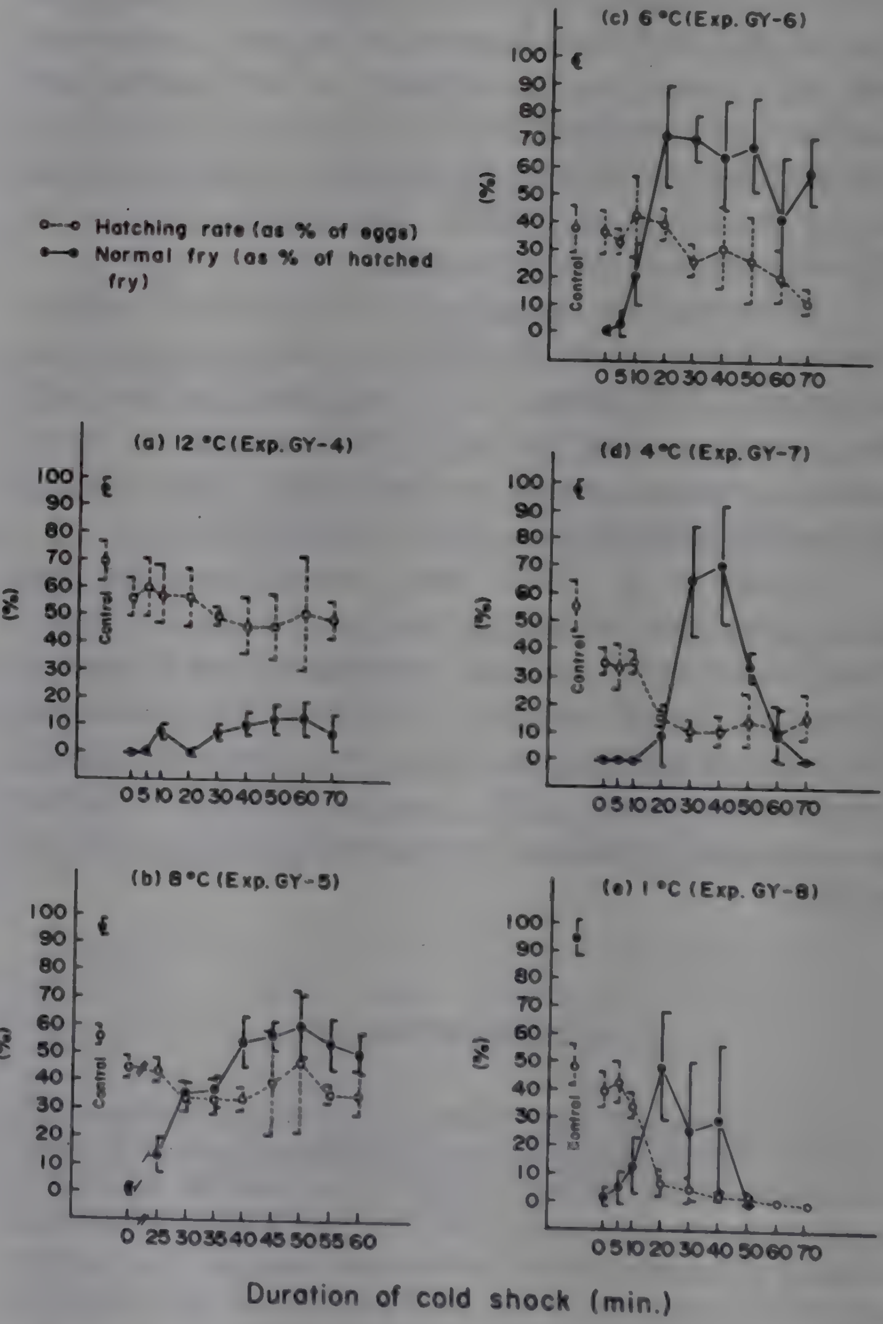

Fig. 4. Mean value and range for hatching rate and percent normal fry of eggs fertilized with UV-irradiated sperm and subjected to cold shocks of various temperature and duration started at 4 min. after fertilization. Controls comprised eggs fertilized with untreated sperm and not subjected to cold shock.

Realized Response of Thai Red Tilapia to Weight - Specific Selection for Growth

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JARIMOPAS, P. 1986. Realized response of Thai red tilapia to weight - specific selection for growth, p. 109-111. *In* J.L. Maclean, L.B. Dizon and L.V. Hosillos (eds.) The First Asian Fisheries Forum. Asian Fisheries Society, Manila, Philippines.

Abstract

Mass selection and weight-specific selection techniques were used to study growth in Thai red tilapia and to determine selection response and realized heritability. In mass selection at the age of 14 weeks, the largest 10% of each population of 500 fish were selected. The growth performance of the selected line was compared to that of the unselected (control) line. In weight-specific selection, fish were selected once at six weeks to have approximately the same standard weight, then were selected a second time based on their growth rate between weeks 6 and 14. Weight and length of the selected line was greater than that of the control in every generation. The selected line was 29.7% heavier and 9.88% longer than the control line after two generations of weight-specific selection. The overall realized heritability estimates were 0.17 for length and 0.19 for weight.

Introduction

Hershberger (1979) suggested that fish genetic improvement programs should focus on improvement of a small number of traits, each of which ought to make the population maximally productive. Falconer (1960) and Kirpichnikov (1981) reported that selective breeding by various means, e.g., mass selection or family selection, can increase production of rainbow trout, common carp and tilapia.

The difficulty of mass (or individual) selection in tilapia is well known and is due primarily to variation in the age of fish in the same generation. Usually offspring are selected for a week or more, and this difference in age, coupled with uncertainty in duration of mouth brooding results in considerable phenotypic size variation among fish that are nominally of the same age. The solution to the problem adapted in this experiment was suggested by Doyle (Doyle and Talbot, in press); it consisted of discarding both large and small fish early in the grow-out period where material effects and uncontrolled phenotypic contributions to size are at their maximum. The remaining fish, ideally of close to identical size, are grown out for an extended period. The differences in size at the end of this second period are used as the selection criteria. The selected trait is weight-specific growth rate, the growth rate of fish all of which start at the same weight. This technique may be especially useful in situations, as in Thailand, where facilities and personnel available for large-scale mass spawning of tilapia are limited.

The Taiwanese have bred red tilapia out of a cross between *Oreochromis mossambicus* and normal *Oreochromis niloticus* (Fitzgerald 1979), yielding first generation offspring, 25% of which were red tilapia. With selective breeding, it took nine years to get 70-80% offspring that grew quickly and were completely orange-red without black spots. In Israel, red tilapia cultured together with normal tilapia of the same generation had low growth rate, apparently because of food competition, most of which was won by the normal tilapia (Chirdchuparnsari 1983).

Thai red tilapia often have black spots and grow slowly. In Thailand application of selective breeding to red tilapia has never been attempted. Selective breeding seems to be a justifiable technique to help improve their growth rate.

Materials and Methods

The experiments were carried out in concrete ponds at the National Inland Fisheries Institute (NIFI) and in cages in Bangsai Ayuthya Province from 21 January 1983 to 4 September 1985. The experiment was conducted in two parts.

Mass Selection of Thai Red Tilapia in Line A

1. Ten females and five males were bred together in five 10-m^2 concrete ponds. Offspring were nursed for six weeks.

2. Two thousand fry from (1) were randomly stocked in four 50-m^2 concrete ponds. At the age of 14 weeks, 25 females and 25 males were taken at random from base population of 500 fish of each pond to serve as control line. Then, individual selection was used; the largest females and males (25 each) were selected from the remaining 450 fish in the base population to serve as the selected line (10% selection).

3. Twenty-five females and 25 males of the same line were reared together in separate 50-m^2 concrete ponds. Eight 5-m^2 concrete ponds were used for four replications.

4. Rearing fish until 7.5 months, F_1 - offspring were produced by mass mating of 25 pairs within each line. Testing procedures applied to the spawners were repeated on the offspring to obtain growth comparisons. The fish population of the control and the selected line at the 14th week when fish showed signs of maturation was measured and discarded.

Weight-Specific Selection of Thai Red Tilapia in Line B

1. Twenty-five pairs of brooders of the unselected (control) line and selected line from line A were bred in eight 50-m^2 concrete ponds, four replications each line. The offspring were nursed for six weeks.

2. Five hundred fry having approximately identical size were selected from (1) and stocked in separate 50-m^2 concrete ponds per line for an additional eight weeks. Both the largest and the smallest fry corresponding to the two tails of the size distribution curve were discarded at week six.

3. The population mean weight and length of 14-week fish before weight-specific selection were measured. The 25 largest females and males were selected by means of 10% mass selection in the selected lines, then cultured separately by sex in two 1 x 1 x 1.5-m^3 cages. Eight cages were used.

4. Procedure (3) was repeated for the fish in the control line ponds, but in this case fish were taken at random.

5. Rearing fish until 7.5 months, females and males of each cage were brought together for mass mating, resulting in F_1 offspring. The subsequent generations were produced by repeating procedures 1 to 5.

6. Fish feed was NIFI tilapia diet. Feeding rate was about 4% daily body weight, twice daily.

7. Length, weight and age of fish were measured on 20% of the population in each pond. Measurements were made on all fish in each cage every 2 weeks to monitor growth rate and regulate the food ration.

8. Selection intensity, selection response and realized heritability of a 14th week fish were determined by the following equations:-

$i = S/\delta$

$h^2 = R/S$ (Falconer 1960)

where i = selection intensity which is equal to the selection differential expressed in terms of standard deviation. S = selection differential, i.e., the difference of mean phenotypic value between selected parents and the population mean; R = selection response, i.e., the differences of mean phenothpic value between the offspring of the selected parents and the whole of the parental generation before selection, or

$R = \Delta_1 - \Delta_2$

where Δ_1 = population mean of corrected offspring (Δ_1 = population mean of selected offspring - population mean of control offspring); Δ_2 = population mean of corrected parents (Δ_2 = population mean of selected parents - population mean of control parents); h^2 = realized heritability; and δ = standard deviation of fish population at 14 weeks before selection.

Results and Discussion

Weight and length of the selected line was greater than that of the control in every generation (Table 1). In F_1 and F_2 generations of line B the selected lines were 21.6% and 29.7% heavier and 5.98% and 9.88% longer than the control line, respectively, after two generations of selection.

Growth comparison of red tilapia after selection in line B is shown in Fig. 1. The growth curves in terms of weight and length show that the selected line grew faster than the control line.

The selection intensity in each generation is given in Table 2. The mean selection intensity in P_0, F_1 and F_2 generations of the selected line B was 1.64, 1.57 and 1.09 by weight and 1.60, 1.39 and 1.28 by length, respectively.

The response to selection can be calculated in two ways: (1) as the difference between the parental and offspring generations after each has been corrected by subtracting the value of the control population or (2) as the difference between the offspring and its contemporaneous unselected control. There seems to be no clear reason to prefer one method of calculation over the other. They can give different results, however, if statistical weighting by degrees of freedom and sample variances is included in the calculation.

The selection response calculated according to method 1 is given in Table 2. At the 14th week, R-values of selected line A were 9.45 g and 1.29 cm. R-values of F_1 and F_2 generations of selected line B were 2.69 g and 1.32 g by weight and 0.46 cm and 0.34 cm by length, respectively.

The cumulative selection response after the second generation of selection calculated according to method (2), is 4.58 g and 0.89 cm, respectively.

Realized heritability using method 1 to calculate R is shown in terms of weight and length in Table 2. Realized heritability of males and females in line A was 0.53 and 0.56 by weight and 0.43 and 0.47 by length, respectively. Average realized heritability of males and females in line B was 0.16 and 0.20 by weight and 0.16 and 0.20 by length, respectively.

The calculation of realized heritability according to method (2) gives the same value without statistical weight. The cumulative selection differential (Table 1) for weight is (27.12-17.27) + (35.8-0-18.29) = 27.36 g and for length is 911.42-9.60) + (12.51-9.75) = 4.58 cm. The corresponding heritability estimates are 0.17 and 0.19.

Selection intensity and selection response of Thai red tilapia in the third generation indicate that the selected fish could grow 29.7% better by weight than the fish of the control line. Weight - specific selection (Doyle and Talbot, in press) is evidently a promising technique for increasing Thai red tilapia growth on the evidence of the observed selection response.

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Table 1. The selection intensity of Thai red tilapia in three generations.

Generation Line	F		Population mean fish ± S.D. Length (cm)	Weight (g)	Mean of selected fish ± S.D. Length (cm)	Weight (g)
A	0 (parent)	♂	10.49 ± 1.41	22.52 ± 8.08	10.90 ± 1.23	25.27 ± 6.80
		♀	9.87 ± 1.25	19.43 ± 8.06	9.98 ± 1.14	20.79 ± 6.80
		$\bar{x}$	10.18 ± 1.38	20.97 ± 8.81	10.44 ± 1.29	23.27 ± 7.07
B	0	♂	9.70 ± 1.34	18.59 ± 6.24	12.03 ± 0.88	31.48 ± 8.75
		♀	9.37 ± 1.09	15.96 ± 5.40	10.77 ± 1.01	22.78 ± 7.24
		$\bar{x}$	9.60 ± 1.14	17.27 ± 6.00	11.42 ± 1.13	27.12 ± 7.85
B	1	♂	9.92 ± 1.91	19.28 ± 11.12	12.84 ± 0.90	36.75 ± 6.86
		♀	9.54 ± 2.06	17.01 ± 11.08	12.39 ± 1.40	34.84 ± 8.33
		$\bar{x}$	9.75 ± 1.98	18.29 ± 11.14	12.51 ± 1.21	35.80 ± 7.87
B	2	♂	10.31 ± 1.31	21.42 ± 7.88	11.78 ± 0.57	28.98 ± 4.27
		♀	9.88 ± 1.19	18.55 ± 6.54	11.30 ± 0.66	26.76 ± 4.38
		$\bar{x}$	9.90 ± 1.28	19.98 ± 7.22	11.54 ± 0.68	27.88 ± 4.43

Table 2. Determination of realized heritability of Thai red tilapia based on length and weight (Method 1)

Generation Line	F		The selection differential length (cm)	weight (g)	The response to selection (R) length (cm)	weight (g)	Heritability (h^2 = R/S) length (cm)	weight (g)
A	1	♂	2.98	19.21	1.28	10.09	0.43	0.53
		♀	2.78	15.60	1.30	8.71	0.47	0.56
		$\bar{x}$	2.88	17.41	1.29	9.54	0.46	0.55
B	1	♂	2.33	12.89	0.46	3.11	0.19	0.24
		♀	1.40	6.81	0.47	2.19	0.34	0.32
		$\bar{x}$	1.84	9.85	0.46	2.68	0.25	0.27
	2	♂	2.72	17.47	0.33	1.35	0.12	0.08
		♀	2.85	17.83	0.31	1.40	0.11	0.08
		$\bar{x}$	2.76	17.51	0.34	1.32	0.12	0.08

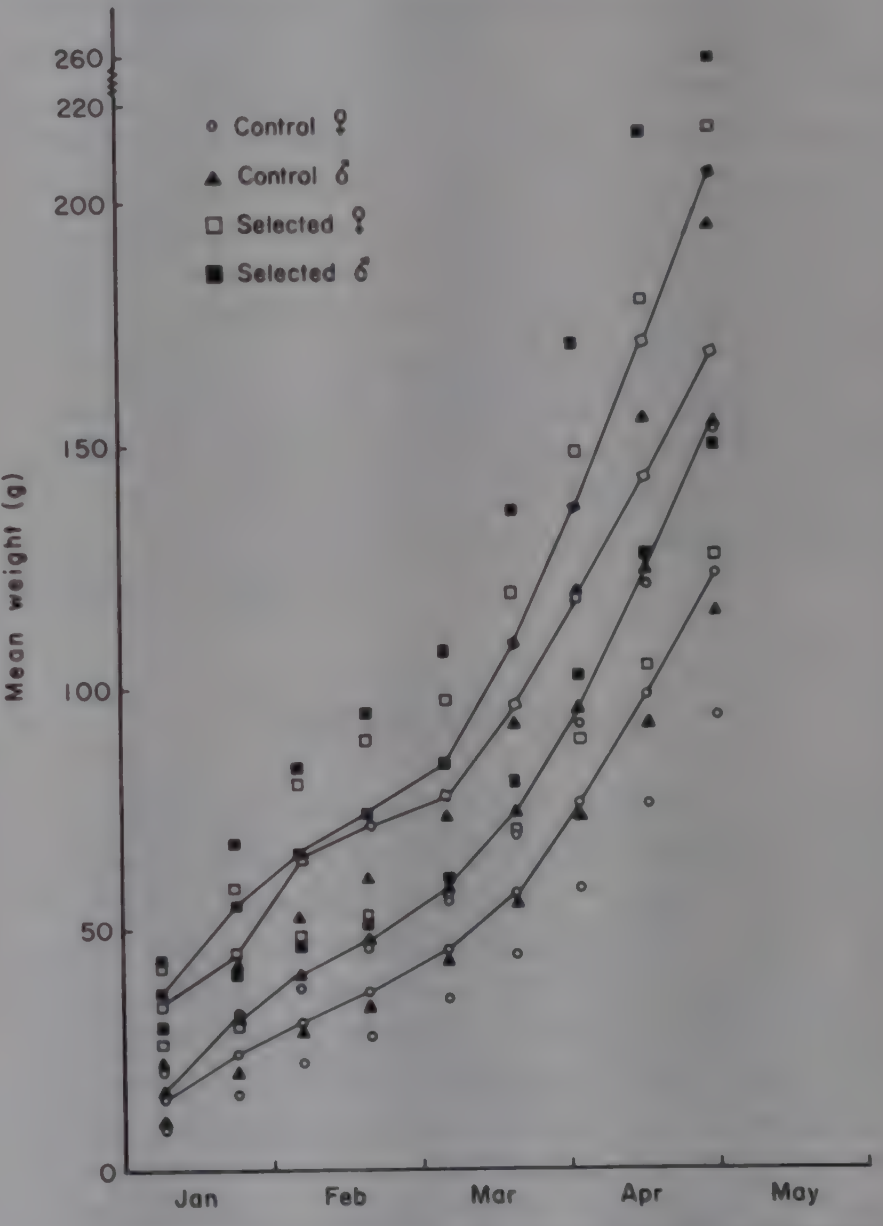

Fig. 1. Mean weight of control and selected tilapia after selection in third generation. Mean values in each group are connected by lines; similar symbols above or below the lines represent one standard deviation.

Hybridization Between Female Red Sea Bream *(Pagrus major)* and Male Crimson Sea Bream *(Evynnis japonica)* by Means of Sperm Cryopreservation

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KUROKURA, H., H. KUMAI and M. NAKAMURA. 1986. Hybridization between female red sea bream *(Pagrus major)* and male crimson sea bream *(Evynnis japonica)* by means of sperm cryopreservation, p. 113-116. *In* J.L. Maclean, L.B. Dizon and L.V. Hosillos (eds.) The First Asian Fisheries Forum. Asian Fisheries Society, Manila, Philippines.

Abstract

Cross breeding between two species of different maturation seasons was attempted by means of sperm preservation. The semen obtained from matured male crimson sea bream *(Evynnis japonica)* was frozen and preserved in liquid nitrogen. DMSO and 0.1 M sodium citrate solution were used as cryoprotectant and extender, respectively. After preservation for 199 days, about 30,000 eggs obtained from matured female red sea bream *(Pagrus major)* were inseminated with 0.2 ml of the semen. Seventy-four per cent of the eggs reached the morula stage, and 69% of the eggs reached the stage of appearance of embryo from those inseminated with preserved crimson red sea bream semen, compared to the values of 89% and 65%, respectively, obtained from eggs which were inseminated with fresh red sea bream semen. Percentage of hatched larvae from the stage of appearance of embryo was 99% in the hybrid, including 4% of malformation, and 100% in red sea bream with no malformation. The mean body weight after the rearing of larval fish for 103 days was 12.6 g in hybrid and 16.2 g in pure red sea bream. Though the growth rate of the hybrid was slow compared to pure red sea bream, it was similar to the wild red sea bream in body color than cultured pure red sea bream. It was anticipated that the market value would be higher in the hybrid than in cultured pure red sea bream.

Introduction

One of the main uses of cryopreservation of fish sperm is to breed new hybrids using preserved sperm. Nevertheless, cryopreservation of fish sperm has contributed little to the development of new hybrids despite considerable efforts. The red sea bream *(Pagrus major)* is the most important cultured marine fish in Japan. However, the difference between the body color of the cultured fish and that of the wild fish is a serious problem because the red color of the fish has important aesthetic implications. The color of the wild red sea bream is preferred-bright red speckled with small blue spots. The cultured fish is dark red with indistinct blue spots. Many efforts, including enrichment of carotenoids in the diet and shade culture, are being made to improve the body color without much success. Kumai and Nakamura (unpublished data) succeeded in hybridizing red sea bream and crimson sea bream *(Evynnis japonica)*. The hybrid had body color similar to that of wild red sea bream and higher growth rate than pure crimson sea bream. However, they noted the difficulty in commercial production of juveniles of this profitable new hybrid because of the time lag in maturation between the two species: the maturation season of red sea bream is in spring while crimson sea bream mature in autumn. The objectives of this study were to examine the conditions for cryopreservation of crimson sea bream sperm, especially with regard to the rate of cooling, and to determine growth rates and coloration of the hybrid juveniles.

Materials and Methods

Semen was collected by hand stripping male crimson sea breams cultured at the Fisheries Laboratory, Kinki University, Uragami, Wakayama. Before collection, mature males were wiped dry around the cloaca. The semen obtained from five males was combined and checked for motility under the microscope. Within 30 min. after semen collection, the semen was diluted with 0.1 M sodium citrate as extender and DMSO as cryoprotectant. The ratio of semen to DMSO and the extender was 0.20:0.15:0.65 by volume. Sperm was frozen in liquid nitrogen vapor by the following method. Polyethylene tubes were used as containers for diluted sperm. The volume of diluted semen in each tube was 0.5 ml. The tubes were placed horizontally on a rack in a styrofoam box (220 x 150 x 180 mm) containing an adequate volume of liquid nitrogen. The cooling rate was regulated by adjusting the distance between the surface of liquid nitrogen and the tubes. The temperature at the middle of the tubes was recorded by thermocouple. After 15 min. the tubes were preserved in liquid nitrogen.

Before the experiments, individual tubes were placed in water bath at room temperature for 30 sec. Motility of the preserved sperm was assessed by the stroboscopic method (Cosson et al. 1985). Thawed semen was prediluted (1:30) using a solution of 0.1 M sodium citrate. After 30 sec., 20μl of prediluted semen was mixed with the same volume of seawater on a slide. A cover glass was put on the suspension immediately. Motility of spermatozoa was photographed with stroboscopic illumination (5 Hz for 2 sec.) and dark-field optics. The sperm suspension was scored for forward swimming speed, percentage of motile spermatozoa and the duration of motility of sperm, considering the time when the suspension was covered with cover glass as the initiation of sperm motility. Pictures to estimate forward swimming speed were taken on an identical field every 15 sec. after initiation of motility (x40 Olympus lens). To calculate the percentage of motile spermatozoa, pictures were taken 5 min. after the initiation (x20 Olympus lens). Flagella movement of the sperm was observed by naked eye in various fields from 5 min. after the initiation. The percentages of motile spermatozoa were estimated from spermatozoa which had flagella movement in the pictures taken 5 min. after the initiation of motility. The duration of the motility was determined as the period from the initiation of motility to the time when less than two spermatozoa were observed in 10 fields.

Red sea bream eggs were collected on 27 May 1985, and a portion of the eggs (33,000) was inseminated with 1 ml (volume after dilution) of preserved crimson sea bream sperm collected and preserved on 21 November 1984. Another portion of the eggs (37,000) was inseminated with fresh red sea bream sperm. The hatched larvae were reared in overland tanks using rotifer *(Brachionus plicatilis)*, copepods and minced *Neomysis* as food.

To compare growth and survival rates of the hybrid and the pure red sea bream, 777 individuals each of hybrid and pure red sea bream were grown in cages in Uragami Bay fed with minced fish.

Results

Fig. 1 shows the freezing curves of the media in tubes placed at distances of 8, 4, 2 and 1 cm from the surface of the liquid nitrogen. The calculated cooling rates between -10°C and -50°C were -21°C/min., -65°C/min., -91°C/min. and -154°C/min., respectively. Fig. 2 shows the linear regression lines obtained by plotting the average forward swimming speed in a picture as a function of time after the initiation of motility. The forward swimming speed of cryopreserved sperm decreased with time. Three replications of motility assessment are summarized in Fig.3 The forward swimming speeds at 60 sec. after the initiation of motility were calculated from the regression lines. Maximum forward swimming speed (134μm/sec.) was recorded in sperm 1 cm above the surface of the liquid nitrogen. Maximum percentage of motile spermatozoa (46.1%) was observed in sperm frozen 4 cm above the surface and maximum duration of motility (114 min.) occurred in sperm frozen 1 cm above the surface. However, the differences in each of three factors in relation to cooling rates were marginal and no clear relationship could be established.

Sperm frozen 4 cm above the surface of liquid nitrogen was mixed with the eggs of red sea bream. Little inferiority in the survival of the hybrid compared to pure red sea bream was observed during embryonic stages (Table 1). The growth rate of juveniles of the hybrid was slightly lower both in fork length and body weight, than that of pure red sea bream (Fig. 4). Mortality during 120 days of culture was 12.1% in the hybrid and 9.5% in the pure red sea bream. The body color was brighter and the blue spots were more distinct in the hybrid than in the red sea bream.

Discussion

The results indicated that none of the motility factors had close relation to the fertilizing capacity of sperm and that there was no clear optimum range of cooling rate for crimson sea bream sperm between 20 and 160°C/min.. Morisawa and Suzuki (1980) reported differences in the environment factors for the initiation and duration of sperm motility such as osmotic pressure and cation content between marine fish and salmonid fish. In addition, it is quite possible that marine fish sperm has higher tolerance to dehydration. If crimson sea bream sperm is considered typical of marine fish sperm in freezing tolerance, it will be rewarding for future advances in cryopreservation technique of fish sperm to investigate the relation between optimum cooling rate and duration of motility and environment factors initiating sperm motility and tolerance to dehydration.

References

Cosson, M.P., R. Billiard, J.L. Gatti and R.Christen. 1985. Rapid and quantitative assessment of trout spermatozoa motility using stroboscopy. Aquaculture 46:71-75.

Morisawa, M. and K. Suzuki. 1980. Osmolality and potassium:the roles in initiation of sperm motility in teleosts. Science 210:1145-1147.

Table 1. A comparison of survival of embryos of hybrid and pure red sea bream.

	Survival (%)		
	*	**	***
Hybrid	77.7	69.0	98.0
Pure red sea bream	88.5	65.3	100.0

* = Percentage of normal developing eggs at morula stage.

** = Percentage of normal developing eggs at the stage of appearance of embryo.

*** = Percentage of hatched larvae from the eggs at the stage of appearance of embryo.

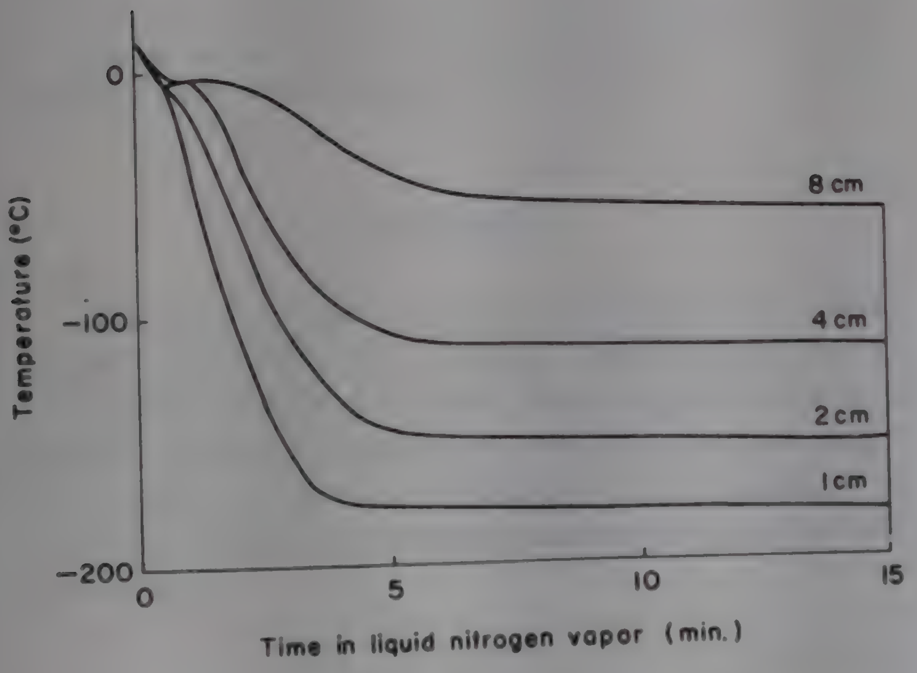

Fig. 1. Freezing curves of the medium in the tubes in accordance with the distance from the surface of the liquid nitrogen. Lengths on each line indicate this distance.

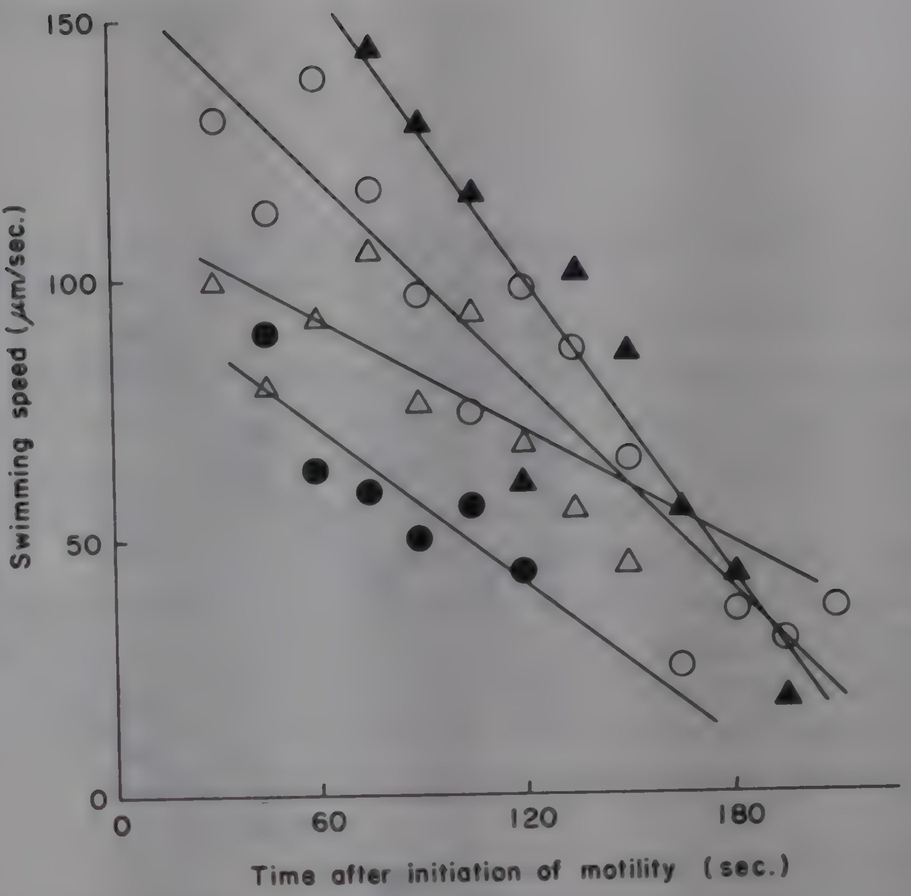

Fig. 2. The relation between forward swimming speed and time after the initiation of motility. ○, ●, △ and ▲ indicate the distances 8, 4, 2 and 1 cm from the surface of the liquid nitrogen, respectively. Lines represent linear regression lines.

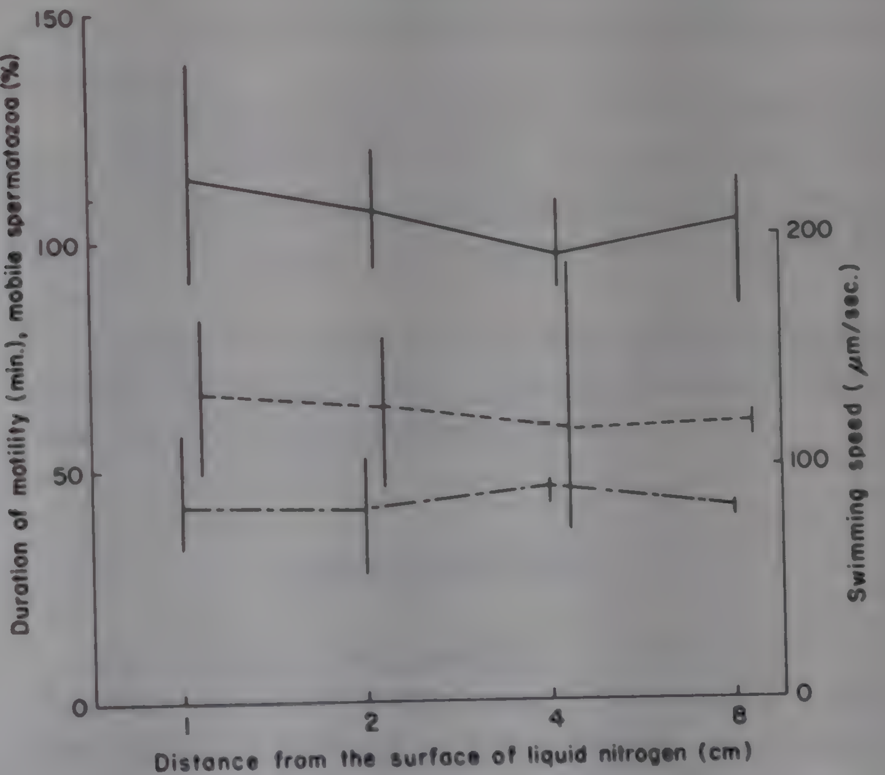

Fig. 3. Comparisons of the motility scores among the preserved sperm frozen at different distances from the surface of liquid nitrogen. Solid line, broken line and chain line represent the duration of motility, forward swimming speed and percentage of motile spermatozoa, respectively. Vertical bars represent the ranges.

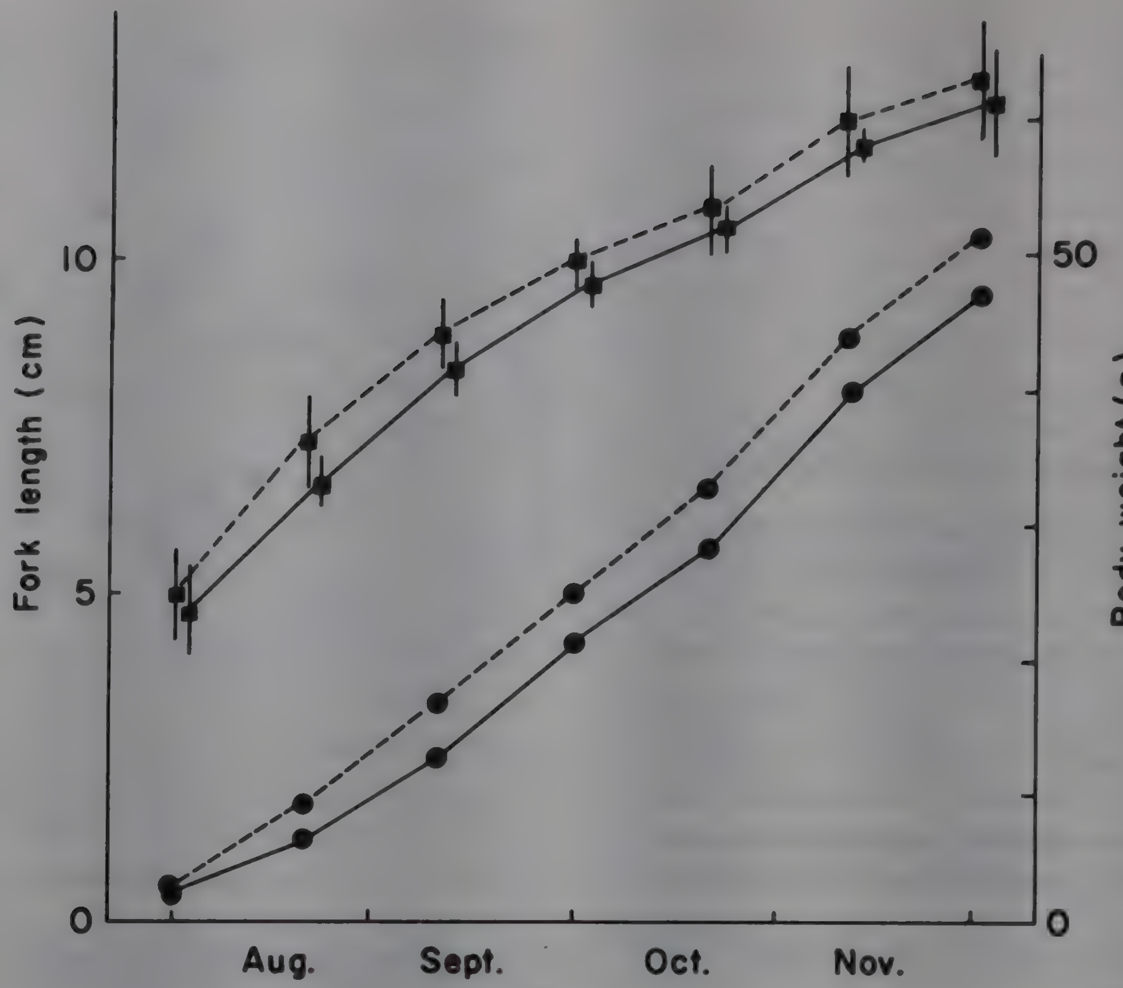

Fig. 4. Comparison of growth between the hybrid (solid line) and the pure red sea bream (broken line). ● and ■ indicate body weight and fork length. Vertical bars represent standard deviation (n = 10). Average body weights were calculated from 100 individuals.

Effects of Different Animal Manures on Fish Farming

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Abstract

This experiment using chicken and pig manure, respectively, for the farming of different sizes of filter-feeding and omnivorous fishes showed that 1 kg of filter-feeding and omnivorous fishes can be produced by using 16.7 kg of chicken manure or 27 kg of pig manure. Chicken manure is especially suitable for the growth of common carp, silver carp and bighead carp with body weight between 50 and 350 g, while pig manure is more suitable for the growth of *Carassius cuvieri*. Delta C analysis was used to prove the close relationship between the feeding habits of silver carp, bighead carp, common carp and *C. cuvieri* and the food base provided by chicken manure directly or indirectly. The study also analyzed the nutrient content of fish flesh in three ponds - chicken-manured, pig-manured ponds and feed-pellet ponds.

Introduction

The dynamic changes in manured ponds and animal manure application in fishponds were studied in an experiment conducted in 1984. With the use of delta C analysis, biological factors in chicken-manured ponds were measured, and the regulation of the changes of matter and energy and their relationship with the fish yield were studied.

Materials and Methods

Chicken and pig manures were used as fertilizers in the experiment. Their components are shown in Table 1. The experiment was conducted in two one-mu (1 mu = 1/15 ha) earthen ponds with water depth of 1.6 m for 202 days from 6 April to 25 October 1984.

Stocked at the ratio of 48:10:14:28 were 1,150 fingerlings of silver carp, bighead carp, common carp and *Carassius cuvieri* (Table 2). The common carp were 'Heyuan' variety provided by the Genetics Department of the Freshwater Fisheries Research Center.

Frequency of manure application depended on air temperature: 2-3 times weekly at high temperature and once a week at low temperature. Average manure application was 3-4% of wet weight of fish, appropriately readjusted according to water color. Water was added to the manure, which was stirred evenly before spreading over the whole pond.

Routine measurements of water temperature, transparency, pH, dissolved oxygen, BOD, phytoplankton, zooplankton, aquatic bacteria, suspension, nitrogen in three forms and inorganic phosphorus were made monthly.

Delta C analysis was used to measure samplings of chicken manure, phytoplankton, bacteria, periphyton, organic detritus, pond humus and fish flesh in the fishpond.

The samples were oven-dried between 90 and 100°C for 10 hours and then powdered. After this pretreatment, MAT-250 Isotope Mass Spectrometer was used to analyze the samples.

Routine measurement of nutrients of bighead carp, silver carp, common carp and *C. cuvieri* farmed in the manure ponds and feed-pellet ponds were made. On 29 June and 11 August, fish samples were measured for growth.

During the experimental period, two fish diseases occurred: *Sinergasilus* and dermoputrescence, which were basically controlled after treatment.

Results and Analyses

The average measurements of physicochemical factors are shown in Table 3 and Figs. 1 and 2. Water temperature was between 19.5 and 30.5°C. As shown in Table 3, the nitrogen content in the pig-manured ponds was comparatively low. According to the measurement by Lei et al. (1983), the inorganic nitrogen content was between 0.97 and 2.06 mg/l in the high yield ponds in Helie Fish Farm, Wuxi, requiring the addition of more manure. The average phosphorus content in the two ponds was equal (Figs. 3 and 4). The ratio between nitrogen and phosphorus was 3.81:1 in the chicken-manured ponds and 1.1:1 in the pig-manured ponds.

Measurements of biological factors are shown in Table 4. The average amounts of phytoplankton, zooplankton, heterotrophic bacteria and total bacteria were higher in the chicken-manured ponds than in the pig-manured ponds, while the average organic detritus was lower in the chicken-manured ponds than in the pig-manured ponds. Changes in all biological factors are shown in Figs. 5 to 8.

The delta C values in the animal tissues and in the feed digested and assimilated were similar. Table 5 shows that the delta C values in common carp and in periphyton and chicken manure were similar; in *C. cuvieri* and in bacteria, organic detritus and plankton between 50 and 120 μm and are relatively similar; values in silver carp and in plankton smaller than 50 μm were most similar; and values in bighead carp and in plankton smaller than 50 μm or larger than 120 μm and were relatively similar.

Measurements of nutrient content in the four fishes from the manure ponds and feed-pellet pond are shown in Table 6. The nutrient content of all the fishes in the chicken-manured ponds was higher than those in the pig-manured ponds, except the protein content of common carp. The fat content of fishes in the chicken-manured ponds was higher than those in the pig-manured ponds. The protein content of bighead carp and *C. cuvieri* was higher, but that of common carp was lower in the manure ponds than in the feed-pellet ponds. The fat content of silver carp and common carp was higher and that of *C. cuvieri* and bighead carp was lower in the chicken-manured ponds than in the feed-pellet ponds. This shows that the nutrient content of fish flesh in the chicken-manured ponds was not lower than that in the feed-pellet ponds, while some of the indices are higher in the chicken-manured ponds than in the feed-pellet ponds. There is no doubt that chicken and pig manures are good fertilizers for fish farming.

Two different sizes of silver carp grew at different rates in different fishponds. In the chicken-manured ponds, more large silver carp survived than in the pig-manured ponds but the fish in the latter were larger at harvest (Table 2).

Survival of small silver carp in the chicken- manured ponds was much better and their average weights were also much higher than in the pig-manured ponds (Table 2).

Small bighead carp survived better in chicken-manured ponds and grew slightly better than in pig-manured ponds. Large bighead carp survived better in the pig-manured ponds but grew better in the chicken-manured ponds (Table 2).

These results show that chicken-manured ponds are more suitable for growing silver carp and bighead carp between 50 and 350 g.

Similarly, Table 2 shows that the pig-manured pond is more suitable for growth of *Carassius cuvieri*.

Only small common carp were stocked and their growth in the chicken-manured ponds was far better than that in pig-manured ponds. Slightly fewer survived in the chicken-manured ponds, but their resulting biomass was more than double that of common carp in pig-manured ponds.

The manure conversion coefficients were 16.7 and 26.99 for wet weight and 5.77 and 5.23 for dry weight of chicken and pig manures, respectively. The average manure cost/kg of fish was 0.66 *yuan* and 0.80 *yuan*. Chicken manure cost was 0.04 *yuan* and pig manure, 0.03 *yuan*. These costs are lower than when fish are farmed with grain and food pellets.

Chicken manure was found to be more suitable for silver carp, bighead carp and common carp, while pig manure was more suitable for *C. cuvieri*. The nutrient content in the flesh of the fish from the manured ponds was by no means worse than that from the feed-pellet pond, while some of the indices of nutrients from the chicken-manured pond are better than those from the feed-pellet pond. Therefore, these two manures are good fertilizers for fish farming.

References

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Table 1. Percentage of components of nutrients in the manures.

Item	Moisture	Total nitrogen	Protein	Fat	Crude fiber	Ash	Lysine
Chicken	45.87	3.25	20.32	6.81	8.19	29.05	0.25
Pig	78.27	2.16	13.47	9.18	11.32	24.20	0.29

Table 2. Stocking and harvesting in the experimental ponds. Upper figures are stocking data; harvest data below.

Item/pond	Chicken-manured ponds						Pig-manured ponds					
Species	LSC	SSC	LBH	SBH	CAC	COC	LSC	SSC	LBH	SBH	CAC	COC
Stocking number (ind./mu)	185	370	40	85	320	150	185	370	40	85	320	150
Size (g/ind.)	321.4	48.9	250	48.2	23.4	6.7	322	48.9	250	48.2	23.4	4.6
Weight (kg/mu)	59.5	18.1	10.0	4.1	7.5	1.0	59.6	18.1	10.0	4.1	7.5	0.7
Total stocking amount (kg/mu)			100.2						100.0			
No. (ind./mu)	182	362	24	82	313	129	146	264	33	62	269	141
Size (g/ind.)	684.3	338.1	452.1	222.0	151.8	282.4	772.9	286.4	439.4	213.7	174.5	111.7
Gross weight (kg/mu)	124.6	122.4	10.9	18.2	47.5	36.4	112.9	75.6	14.5	13.3	47.0	15.8
Net weight (kg/mu)	65.1	104.3	0.85	14.1	40.0	35.4	53.2	57.5	4.5	9.2	39.5	15.1
Survival rate (%)			95%						80%			
Total gross yield (kg/mu)			359.9						278.9			
Total net yield (kg/mu)			259.7						178.9			
Weight increase rate (times)			3.59						2.79			

LSC = large silver carp, SSC = small silver carp, LBH = large bighead carp, SBH = small bighead carp, CAC = *Carassius cuvieri* and COC = common carp

Table 3. Physicochemical factors in the fishpond.

Item	Chicken-manured ponds	Pig-manured ponds
Transparency (cm)	28.83 ± 4.58	31 ± 7.85
Dissolved oxygen (mg/l)	4.53 ± 1.52	4.94 ± 0.93
BOD (mg/l)	5.2 ± 1.77	4.23 ± 0.93
Inorganic nitrogen (mg/l)	1.41 ± 0.86	0.4 ± 0.38
Inorganic phosphorus (mg/l)	0.37 ± 0.45	0.37 ± 0.57
pH	6 ~ 8.2	5.8 ~ 8.3

Table 4. Biological factors in the fishpond.

Item	Chicken-manured ponds	Pig-manured ponds
Phytoplankton (mg/l)	40.44 ± 9.48	30.21 ± 16.95
Zooplankton (mg/l)	12.41 ± 8.35	7.03 ± 5.67
Total suspension (mg/l)	260.02 ± 33.82	284.5 ± 33.73
Organic detritus (mg/l)	208.37 ± 21.98	247.26 ± 26.79
Total bacteria (individual/ml)	$5.95 \times 10^6 \pm 3.94 \times 10^6$	$5.1 \times 10^6 \pm 3.65 \times 10^6$
Heterotrophic bacteria (individual/ml)	$8.15 \times 10^3 \pm 6.33 \times 10^3$	$2.33 \times 10^3 \pm 1.02 \times 10^3$

Table 5. Delta C values in chicken-manured ponds.

Item	Delta C value
Chicken manure	−18.22
Periphyton	−19.38
Common carp	−19.43
Bacteria	−20.62
(Carassius cuvieri)	−20.89
Organic detritus	−21.12
Plankton between 50 and 120 μm	−22.36
Plankton smaller than 50 μm	−23.02
Pond mud	−23.13
Silver carp	−23.56
Bighead carp	−24.25
Pond humus	−24.62
Plankton larger than 120 μm	−25.63

Table 6. Nutrient contents in fish flesh (%).

Item	Chicken-manured ponds	Pig-manured ponds	Feed-pelleted ponds
Silver carp			
moisture	73.47	78.04	74.30
fat	6.17	1.84	5.40
protein	20.02	19.09	20.06
Bighead carp			
moisture	78.37	80.20	77.60
fat	0.96	0.42	7.82
protein	19.56	18.80	14.31
Common carp			
moisture	78.03	77.80	72.85
fat	3.86	0.49	2.08
protein	18.25	21.25	24.82
Carassius cuvieri			
moisture	74.01	78.31	77.07
fat	3.83	1.28	5.78
protein	21.37	19.22	17.77

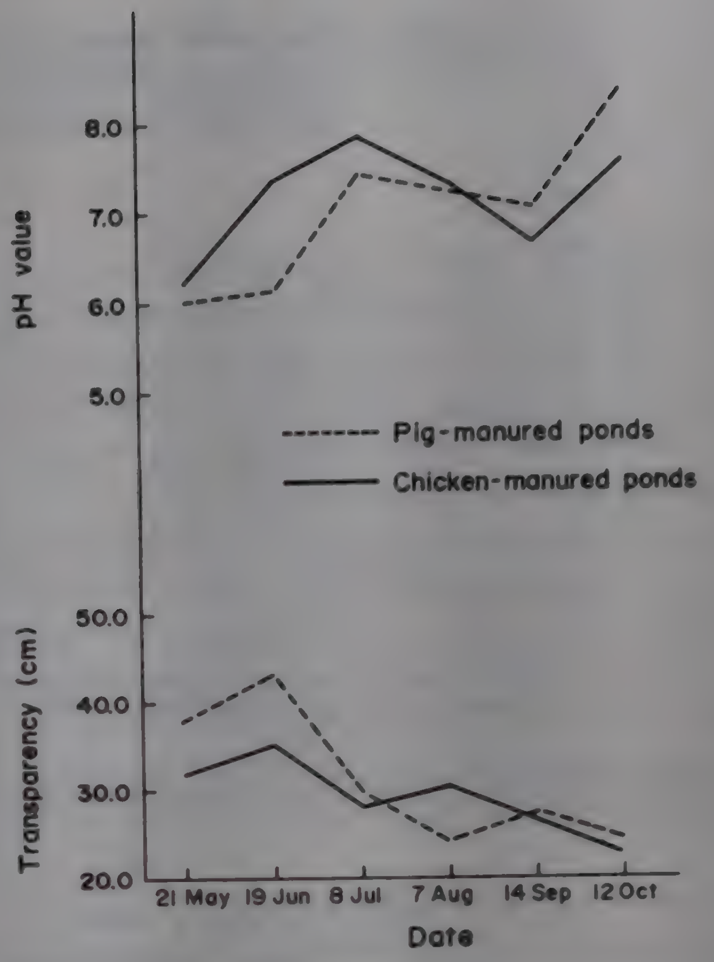

Fig. 1. Fluctuations of pH and transparency in experimental ponds.

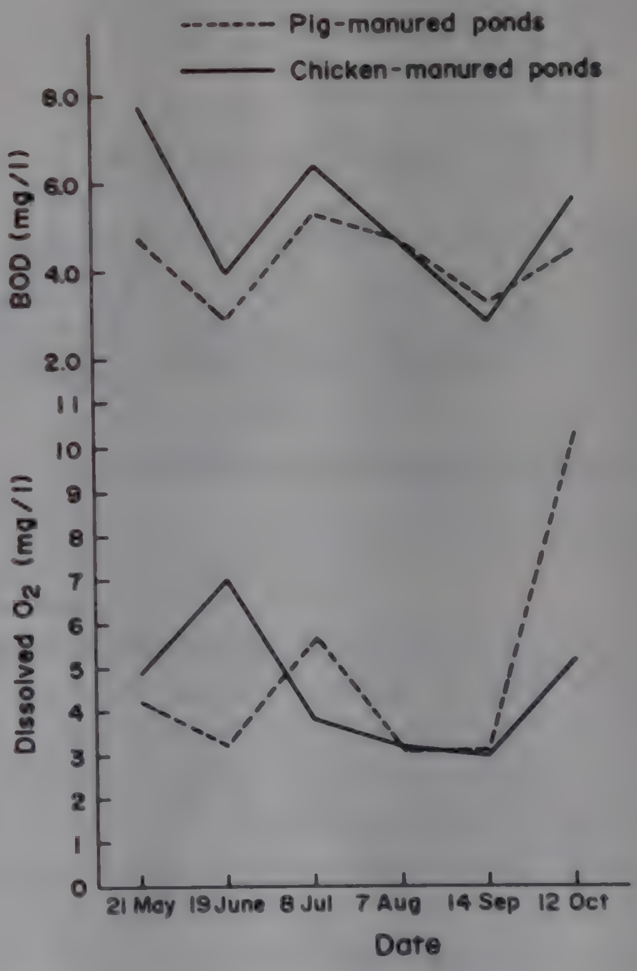

Fig. 2. DO and BOD levels in experimental ponds.

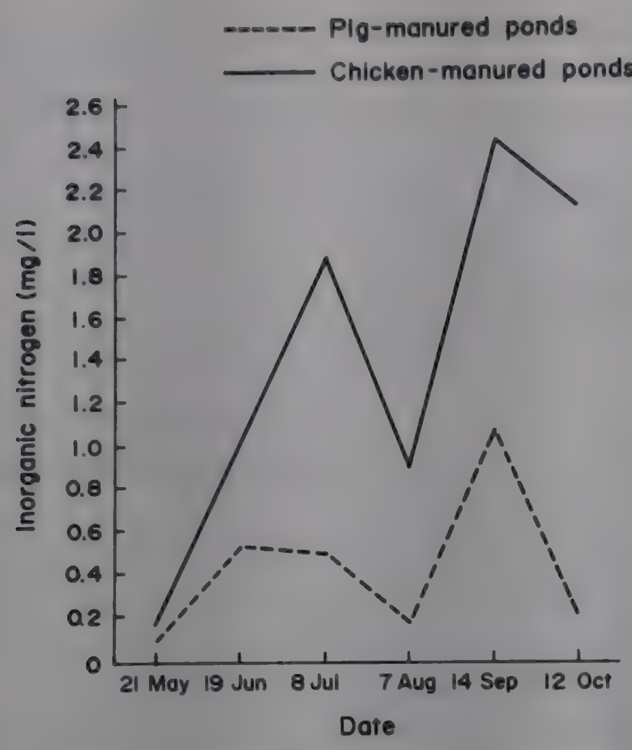

Fig. 3. Inorganic nitrogen levels in experimental ponds.

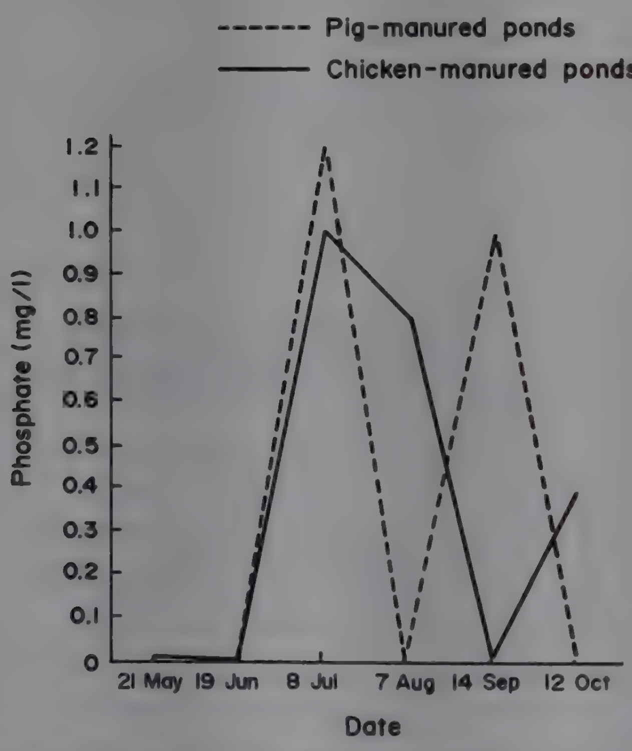

Fig. 4. Phosphate levels in experimental ponds.

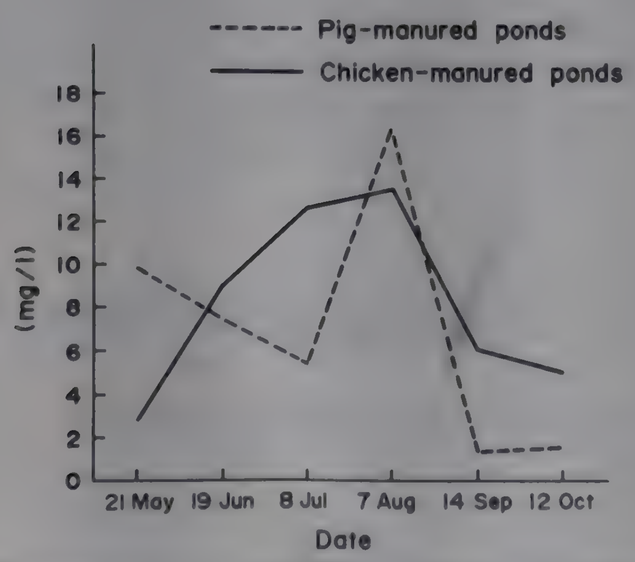

Fig. 5. Fluctuations of zooplankton biomass in experimental ponds.

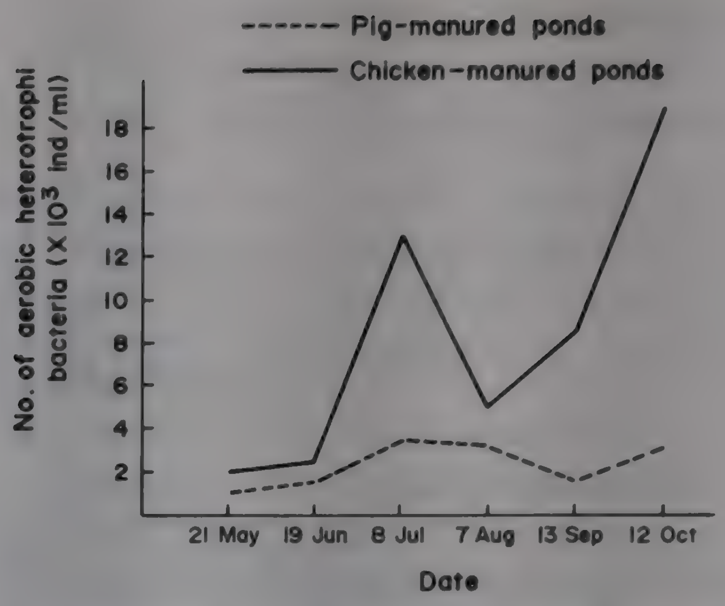

Fig. 6. Fluctuations of the number of aerobic heterotrophic bacteria in experimental ponds.

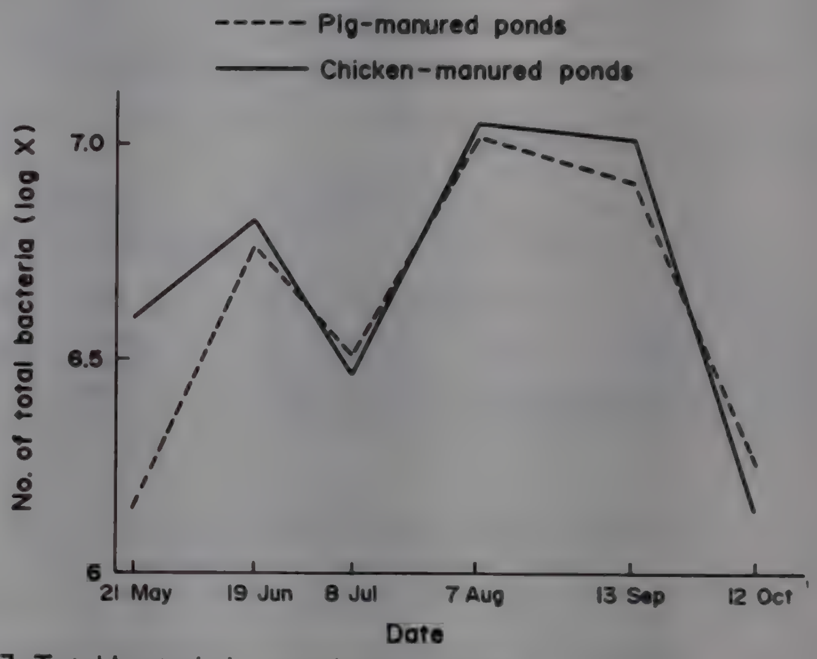

Fig. 7. Total bacteria in experimental ponds.

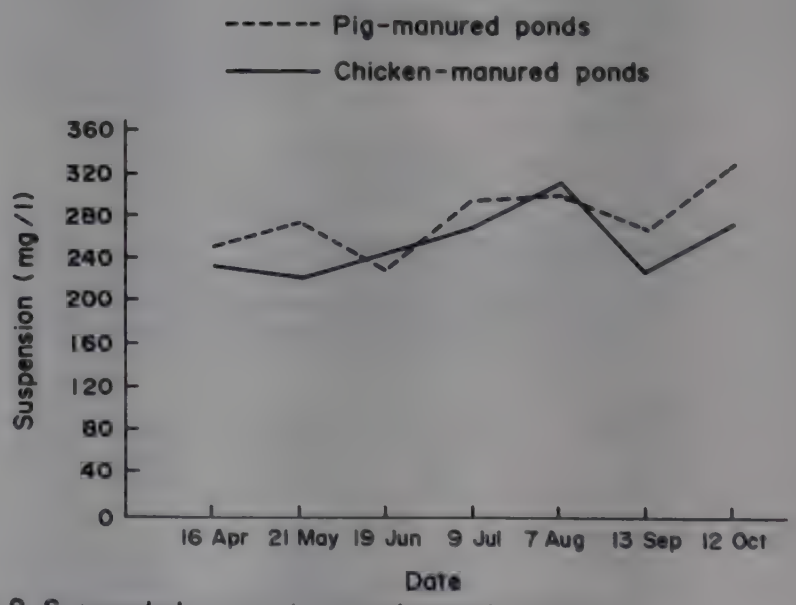

Fig. 8. Suspended matter in experimental ponds.

Effects of Fertilizers and Feeds as Nutrient Sources on *Oreochromis niloticus* Production in Philippine Brackishwater Ponds

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Abstract

An experiment was conducted to test the effect of chicken manure, 16-20-0, feeds with 20% crude protein (CP) and their combinations on pond productivity and water quality and on the growth and production of Nile tilapia *(Oreochromis niloticus)* in brackishwater ponds. Results showed low average daily weight increments of Nile tilapia of 0.52 to 1.25 g/day and 0.56 to 1.04 g/day for the first and second runs, respectively. Fish from treatments that received feed (either alone or in combination with chicken manure and/or 16-20-0) were significantly bigger ($P < 0.01$) than fish from treatments without feed. The combination of chicken manure and 16-20-0 did not contribute significantly to the production of tilapia. Higher temperatures were obtained in the treatments with inputs. Dissolved oxygen content was lowest in the feed-chicken manure-16-20-0 combination. Nitrate and nitrite levels were significantly higher in the treatments that received chicken manure ($P < 0.05$); available phosphorus was significantly highest ($P < 0.01$) in the chicken manure-feed combination. There are indications that the phosphorus content of chicken manure increased that in the soil although total phosphorus in the soil contributed only about 0.8% of that in water. The organic matter content of the pond soil was influenced by the various inputs resulting in significantly higher ($P < 0.01$) organic matter content of the sediments in the treatments that received chicken manure, feeds and their combination. There were no significant differences ($P > 0.05$) among the treatments in terms of primary productivity, zoo- and phytoplankton populations and algal biomass. A highly significant difference ($P < 0.01$) among the treatments was observed in terms of cholorophyll *a* concentration.

Introduction

In the Philippines, fertilization and feeding are common practices in brackishwater pond culture. Tang (1979) described the different levels of management intensity for the farming system based on natural food production. Several studies attempted to determine the production potential of ponds (Lovshin and Da Silva 1975; Collins and Smitherman 1978; Hepher and Pruginin 1982). Hepher and Pruginin (1982) demonstrated an increase of 3.4 fold production of *Oreochromis niloticus* using superphosphate; 1.7-fold with cow manure; and 3.3-fold with poultry manure over natural production. With the combination of superphosphate and cow manure, production increased fivefold. In brackishwater, using all-male *Oreochromis mossambicus* and 10,000/ha stocking density, significant fish production was attained when the ponds were fertilized with pig manure (Fortes et al. 1980; Tamse et al. 1985).

The above works are all yield trials so that what happened to the inputs and their effect on fish production and pond productivity is not very well known. Thus, the specific objectives of this study were: (1) to determine the effect of selected physical, chemical and biological parameters on the production of Nile tilapia and (2) to determine the effects of the various inputs and their combinations on water quality and pond productivity.

Materials and Methods

The experiment was carried out at the Brackishwater Aquaculture Center (BAC), College of Fisheries, University of the Philippines in the Visayas, Leganes, Iloilo, Philippines. The first run was on 24 November 1984-24 April 1985 and the second run on 3 October 1985-3 March 1986. Twenty-one units of 500-m^2 ponds were used. Agricultural lime and chicken manure were initially applied at the rate of 2 t/ha. Water was maintained at about 60-cm depth.

Nile tilapia fingerlings were taken from freshwater ponds in Hacienda Binitin, Murcia, Negros Occidental, Philippines, and transported in oxygenated 60-l plastic bags to BAC at Leganes, Iloilo. At BAC, they were transferred to aerated tanks and acclimated to saline water by increasing the salinity to approximately 5 ppt daily until 27 ppt was attained.

Seven treatments with three replicates each were tested in a randomized complete block design (RCBD) as shown in Table 1.

Feeding rate for the first month of culture was based on the average weight of the fish in all ponds at day zero. The amount of feed was adjusted every month after each sampling period. Feed composition was: rice bran, *ipil-ipil (Leucaena leucocephala)* leaf meal, corn meal, fishmeal, soybean meal, vegetable oil, vitamin premix and shrimp head meal.

Fish were stocked at an average of 7.9-9.2 g and 43.3-47.2 g (first and second runs, respectively). Samplings were done every month thereafter. Fish were harvested after 151 days of culture.

Daily measurements of temperature with a Taylor minimum-maximum thermometer and visibility by means of Secchi disk were made daily. Dissolved oxygen, pH and salinity were measured thrice weekly by means of YSI model 51 B oxygen meter, Atago Tanaka S-100 refractometer, and Corning pH meter model 10, respectively. Ammonia nitrogen (NH_3), nitrate (NO_3), nitrite (NO_2), and available phosphorus (P) were determined four times a month by chlorometric method (Strickland and Parson 1972) and iron by ammonium acetate extraction method. Water samples for phytoplankton and zooplankton were taken four times a month, twice before draining and twice after flooding. Concentrates of water samples were made and preserved in Lugols solution. Zooplankters were identified and counted with a Sedgewick Rafter counting chamber and cell and a hemocytometer was used for counting phytoplankton.

Organic matter and soil pH were measured twice a month before draining. Organic matter was analyzed by the Chromic method (Walkley and Black 1965). *Lablab* samples were collected with a 250-ml plastic sampling bottle with cut-off bottom. *Lablab* production was determined by the method described by Baldevarona (1979).

Primary productivity was estimated by the open-water method and chlorophyll *a* concentration with the procedures described by Lind (1974).

All statistical analyses were performed with the use of the SYSTAT analysis package.

Results and Discussion

The average weight increment of Nile tilapia was 0.52-1.26 g/day and 0.56-1.04 g/day for the first and second runs, respectively (Table 2). The average size in the treatments that received feed, either alone or in combination with organic and/or inorganic fertilizer, was significantly greater ($P < 0.01$) than in the other treatments. This slow growth could have been due to the poor quality of the tilapia stock which electrophoretic analysis showed to be with 37% admixtures of *Oreochromis mossambicus* alleles. Further, despite the low resulting density in the ponds due to high mortality, the daily weight increment was low. The combination of chicken manure and 16-20-0 did not significantly improve the daily weight increment which was 32-37% over the daily weight increment of the fish from the treatment with input. Tang (1979) estimated that fish production can be increased by at least six times by fertilization alone and 18 times with feed added. Results of these trials did not approach previous estimates, but showed that under experimental conditions, fertilization, feeding and feeding/fertilization increased yield by 14.5 to 40%; 48 to 69%; and 50 to 71%, respectively.

Two very important physical parameters that registered significant differences among the various treatments are temperature and Secchi disk visibility. Despite the very narrow differences in water temperature among the various treatments, significant differences ($P < 0.05$) were noted. Temperature increased in the treatments that received added inputs regardless of their type (Table 3). It appears that soils with inputs, particularly chicken manure and feeds, behaved like organic matter-amended soils where heat was liberated, increasing temperature (National Research Council 1976; Watanabe 1984).

Secchi disk visibility was significantly lower ($P < 0.01$) in treatments with inputs and highest in the treatments without input (Table 1). The lowest visibility readings were in the treatments with feed, indicating that feed served not only as fish food but also as source of nutrients for aquatic microorganisms, such as phytoplankton, that contribute to productivity.

Table 4 presents the means of selected water chemical parameters. The treatments with feed were significantly ($P < 0.01$) lower in dissolved oxygen content than those without. Feed, chicken manure and 16-20-0 recorded the lowest dissolved oxygen among all treatments. Tamse et al. (1985) observed that the morning and afternoon oxygen saturation levels of manured ponds decreased two days after the start of application. The nonmanured ponds, however, had higher levels of oxygen saturation. Feeds and chicken manure, being organic matter, withdrew large quantities of oxygen upon decomposition (Matida, unpublished data).

The nitrates in the treatments with chicken manure were significantly higher than those without ($P < 0.05$). Nitrites, however, were significantly higher in treatments I, V and VI which also received chicken manure. Available phosphorus was also significantly highest ($P < 0.01$) in the treatments with chicken manure, 0.24%. Thus, addition of either or both would contribute to the phosphorus in the water. This is particularly true for those not completely utilized by the fish. The N, P and S are main constituents of organic compounds and these are liberated from organic materials during the formation and dynamics of soil organic matter by microbial activity (Flaig 1984).

Soil phosphorus differed significantly among the treatments ($P < 0.05$) but was significantly highest in Treatment V where it could have been contributed by the chicken manure and feed. The P content of chicken manure resulted in higher P content of the pond soil although the total P in the soil contributed very little P to the water. The concentration of available P in the water was only about 0.8% of the total P in the soil (Tables 4 and 5). In general, higher available P in the water was observed where total P in the soil was high.

The organic matter content of the sediments was significantly different among the treatments ($P < 0.01$). In the first run, highest percentage of organic matter in the sediment was observed in Treatment I with chicken manure only while the lowest was in the treatment without input. In the second run, the highest organic matter contents were observed in the treatments with feed. There was indication that organic matter influenced dry pH, that is, dry pH values decreased as the percentage of organic matter increased. It is possible that the increase in acidity of the sediment resulted when organic matter decomposed under anaerobic condition, releasing organic acids (Watanabe 1984).

No significant differences were observed among primary productivity, zooplankton and phytoplankton populations and *lablab* or algal biomass. A highly significant difference, however, was among the treatments in terms of chlorophyll *a* concentration. A direct relationship was observed between primary productivity or the amount of carbon produced and the amount of chlorophyll *a* concentration. The same observation was made by Fortes (1973) in his studies of chlorophyll in the waters of selected manmade ponds in Alabama. The treatments with the combination of chicken manure, feed and/or 16-20-0 had the highest primary production in both the first and second runs. Similarly, the concentrations of chlorophyll *a* in water were highest in the treatments with the combination of chicken manure feed and/or 16-20-0. No clear relationship was observed between the plankton and *lablab* biomass although the highest densities of zooplankton were observed in the combination treatment of chicken manure, feed and/or 16-20-0. Although no trend was established on the effect of the different treatments on zooplankton, phytoplankton and *lablab* biomass, it was very clear that the different inputs increased the various parameters because the treatment without input consistently gave the lowest values.

As overall effect of the added inputs, the average weights attained by the tilapia were highest in the treatments that received feed, either alone or in combination with the other inputs. The sizes of the fish at harvest from the treatments that received feed were not significantly different ($P > 0.05$), 87.1-189.4 g and 83-203.4 g for first and second runs, respectively, but were significantly different from the treatments without feed ($P < 0.01$). Table 6 shows that available P was highest in treatments V and VI. Chlorophyll *a* concentration and zooplankton population were also higher in these treatments than in the treatments without feed. However, nitrite concentration in the treatments that received feed-fertilizer combinations were high, although not at the level (0.5 mg/l) toxic to the fish (Boyd 1979). Feeds served not only as food but also as nutrient sources similar to fertilizers. Boyd (1982) computed the amount of N and P added into fishponds as feed and concluded that nutrients from feeds supplied on a continuous basis are more effective in promoting plankton growth than nutrients from fertilizers applied at two- to four-week intervals.

In terms of total inputs, Treatment VI had the highest (102.5-401.1 kg/ha/week), followed by Treatment V (90-399.6 kg/ha/week), Treatment III (32.4-365 kg/ha/week), Treatment I (57.6 kg/ha/week) and Treatment II (12.5 kg/ha/week). Phosphorus added into the ponds was equivalent to 3.061-6.401 kg/ha/week, 0.561-3.901 kg/ha/week, 2.736 kg/ha/week, 0.325-3.665 kg/ha/week, 2.5 and 0.236 kg/ha/week, respectively. These results show that the use of combined organic and inorganic fertilizers plus feed may give the highest yield but could be wasteful and expensive. Combinations of chicken manure + 16-20-0 did not significantly increase the growth and production of the tilapia while chicken manure and feed combined provided high phosphorus values that increased pond productivity and fish production.

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Table 1. The seven treatments used in experiments. Three replicates of each were performed.

Treatment	Inputs	Rate/mo.	Interval of application/ week
I	Chicken manure only	57.6 kg/ha	thrice
II	16-20-0 only	12.5 kg/ha	once
III	Feeds only (20% crude protein)	6% of fish biomass	6 times
IV	Chicken manure + 16-10-0	57.6 kg/ha 12.5 kg/ha	thrice once
V	Chicken manure + feeds (20% crude protein)	57.6 kg/ha 6% of fish biomass	thrice 6 times
VI	Chicken manure + 16-20-0 + feeds (20% crude protein)	57.6 kg/ha 12.5 kg/ha 6% of fish biomass	thrice once 6 times
VII	No input		

Table 2. Summary results for *Oreochromis niloticus* showing the averages of three replicates of each treatment.

Treatment	Initial wt (g) (1)	(2)	Harvest wt (g) (1)	(2)	Survival (%) (1)	(2)
I	9.2	47.2	126.9	114.1	11.4	39.5
II	9.0	43.2	122.8	101.6	14.1	28.8
III	8.9	45.5	189.4	203.2	13.9	38.4
IV	9.2	44.8	111.0	114.6	20.1	31.9
V	9.1	45.0	178.5	200.2	14.8	47.2
VI	9.1	43.3	200.2	179.4	16.4	45.7
VII	7.9	43.3	87.1	83.0	17.0	31.2

Table 3. Means of the selected water parameters during the 151-day culture of *O. niloticus*.

Treatment	Temperature (°C)[a] 1st run	2nd run	Depth (cm) 1st run	2nd run	Secchi disk visibility (cm)[b] 1st run	2nd run
I	26.0b	25.0a	65.2	64.6abc	41.9ab	30.3bc
II	26.0b	24.8a	66.6	58.6a	43.3b	31.6c
III	25.9b	25.1ab	61.9	68.6bc	41.2ab	24.3a
IV	25.9b	24.9a	66.6	65.4abc	41.1ab	27.6b
V	25.8ab	25.0a	61.5	61.8ab	39.5ab	23.0a
VI	25.8ab	25.0a	60.4	66.8abc	37.2a	23.2a
VII	25.7a	25.4b	60.1	73.3c	44.1b	33.9c
	*	**	n.s.	**	**	**

Legend: I – Chicken manure; II - Inorganic fertilizer (16-20-0); III – Feed; IV – Chicken manure + 16-20-0, V – Chicken manure + feed; VI – Chicken manure + feed + 16-20-0; VII – No input. Values with same letters or asterisks are not significantly different.

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Table 5. Averages of selected parameters of the sediments of the various treatments.

Treatment	Fe (mg/l) 1st run	2nd run	P (mg/l) 1st run	2nd run	Organic matter 1st run	2nd run	pH Dry 1st run	2nd run	pH Wet 1st run	2nd run
I	221.4	155.6	20.7b	15.4	4.5a	3.6a	6.8	6.5bc	7.1	7.4c
II	233.3	196.4	19.7ab	19.6	4.1ab	3.5a	6.9	6.5c	7.2	7.2ab
III	252.6	210.7	16.4a	14.0	3.6bc	4.4ab	6.3	6.3abc	7.2	7.3bc
IV	225.7	176.7	18.8ab	12.9	3.5bc	3.8ab	6.3	6.4abc	7.3	7.1a
V	259.3	219.7	21.2b	19.0	3.4bc	4.4ab	6.4	6.3abc	7.2	7.2ab
VI	203.6	228.3	19.3ab	16.9	3.5bc	4.7b	6.3	6.1a	7.1	7.3bc
VII	163.0	139.4	18.0ab	20.4	3.0c	3.9ab	6.6	6.2ab	7.1	7.3bc
	n.s.		**		**	**	**	*	n.s.	**

Legend: I – Chicken manure; II – Inorganic fertilizer (16-20-0); III – Feed; IV – Chicken manure + 16-20-0; V – Chicken manure + feed; VI – Chicken manure + feed + 16-20-0; VII – No input. Values with same letters or asterisks are not significantly different.

Table 6. Averages of variables significantly different among treatments in a two-way analysis of variance.

Treatment	Fish production (kg/ha)	NO_2 (ppm)	Available P (mg/l)	Chlorophyll a (kg/l)	Zooplankton (ind/l)	Temperature (°C)	O.M. (%)	Depth (cm)
I	517.4	0.022	0.107	33.680	980	25.06	3.7	65.2
II	394.3	0.020	0.046	27.86	799	24.89	3.3	57.9
III	936.6	0.034	0.137	36.665	1,158	25.16	4.6	69.4
IV	435.4	0.30	0.147	37.10	938	25.04	3.88	66.07
V	1,157.6	0.034	0.230	43.32	1,304	25.02	4.4	64.5
VI	982.7	0.044	0.183	46.78	1,378	25.11	4.61	67.3
VII	310.9	0.020	0.021	27.42	883	25.38	3.95	74.0

Legend: I – Chicken manure; II – Inorganic fertilizer (16-20-0), III – Feed; IV – Chicken manure + 16-20-0; V – Chicken manure + feed; VI – Chicken manure + feed + 16-20-0; VII – No input.

Table 4. Means of selected chemical parameters of the various treatments.

Treatment	D.O. (mg/l) 1st run	2nd run	pH 1st run	2nd run	Salinity 1st run	2nd run	NH_3 (mg/l) 1st run	2nd run	NO_3 (mg/l) 1st run	2nd run	NO_2 (mg/l) 1st run	2nd run	Avail. P (mg/l) 1st run	2nd run
I	4.00cd	4.64b	8.06	7.70c	35.80ab	30.74	0.043	0.076	0.564ab	0.360	0.023b	0.021abc	0.21b	0.113ab
II	4.46de	5.45c	8.02	7.82c	35.61a	30.81	0.032	0.058	0.585ab	0.344	0.016a	0.018a	0.055a	0.042a
III	3.46b	3.52a	8.04	7.44ab	36.02bc	30.55	0.039	0.056	0.468a	0.327	0.016a	0.032bcd	0.71a	0.117ab
IV	3.98c	4.59b	8.12	7.67c	35.96abc	30.37	0.037	0.071	0.656b	0.390	0.017ab	0.029bcd	0.142b	0.147bc
V	3.10ab	2.80a	8.08	7.38a	36.1bc	30.97	0.042	0.066	0.622b	0.414	0.020ab	0.034cd	0.212c	0.236c
VI	2.97a	2.99a	8.09	7.41ab	36.1bc	30.77	0.037	0.068	0.657b	0.343	0.023b	0.041d	0.216c	0.184bc
VII	4.53e	5.31bc	8.76	7.61bc	36.20c	30.16	0.030	0.085	0.589ab	0.326	0.014a	0.019ab	0.059a	0.034a
	**	**	n.s.	**	*	n.s.	n.s.		*	n.s.	*	**	**	**

Legend: I – Chicken manure; II – Inorganic fertilizer (16-20-0); III – Feed; IV – Chicken manure + 16-20-0; V – Chicken manure + feed; VI – Chicken manure + feed + 16-20-0; VII – No input. Values with same letters or asterisks are not significantly different.

A Preliminary Study on Sources of Fish Growth in Manured Ponds Using Delta C Analysis

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Abstract

In a manured fishpond, the flow of organic matter from manure to feeds to fish is a complicated process of pond dynamics. In order to understand better and possibly manipulate this process and to help us improve farming productivity, we introduced stable carbon isotope analysis (delta C) in our experiment. We measured delta C of the fish, fish feeds, manure and sediments, and thus traced the flow of carbon, analyzing the interactions of fish growth and the complicated food web in a manured pond. The data showed that pelagic filtering and omnivorous bottom feeding species obtained carbon from both autotrophic and heterotrophic production systems. This is of practical significance in planning aquacultural activities and investigating pond dynamics.

Introduction

Organic manure, such as animal wastes, is often used in ponds of integrated fish farming. Between the input of manure and output of fish come a series of complicated processes involving food organisms, material circulation and energy conversion. This is generally termed pond dynamics. To raise farming productivity, it is useful to understand these processes. In recent years, scientists have been studying pond dynamics with various methods (Buck et al. 1978; Schroeder 1978; Guo 1983; Hu 1983; Li 1983), including carbon isotopic trace analysis. This analysis indicates the relationship between the food and fish growth and may provide information not revealed by the conventional method of microscopic analyses of fish intestine contents (Schroeder 1983a; Shan et al. 1985).

It is well known that carbon is the major element in nutrition of organisms and the principal constituent, accounting for 50% of organisms by dry weight.

The carbon of organic and inorganic matter contains two stable isotopes: ^{12}C and ^{13}C. The ^{13}C accounts for about 1% of the natural abundance. The ratio of $^{13}C{:}^{12}C$ provides a naturally occurring tracer. The ratio of $^{13}C{:}^{12}C$ is reported as delta C, where:

$$\text{Delta C} = \left(\frac{^{13}C/^{12}C \text{ sample}}{^{13}C/^{12}C \text{ standard}} - 1 \right) \cdot 1{,}000 \text{ (per mil)}$$

(The standard sample of delta C PDB is a carbonate rock from South Carolina, USA.)

The distribution of these isotopes in natural carbon reflects in the composition of atmospheric CO_2 as $^{13}CO_2$. During photosynthesis, plants metabolize the atmospheric CO_2 in two main pathways of photosynthesis: C_3 circulation and C_4 circulation. Delta C of these two types of plants are measurably different (Smith and Epstein 1971). Unlike plants that have a selectivity in metabolizing ^{13}C or ^{12}C, an animal's delta C is decided by delta C in the food. The isotope C incorporated into the fish tissues is equal to that of the metabolized food (De Niro and Epstein 1978; Schroeder 1983), that is, delta C of fish is approximately equal to delta C in food. Based on this similarity, we adopted the mass spectrographic analysis of the isotope ratios to trace the flow of carbon by accurately sampling the delta C index of fish, food and manure of a given pond.

Materials and Methods

The earthen pond area in our experimental farm was one mu (1/15 ha) with water depth of 1.5-2 m filled with Lake Taihu water. According to the ratio of 9:2:6:3, two-year old silver carp *(Hypophthalmichthys molitrix)*, bighead *(Aristichthys nobilis)*, baiji *(Carassius cuvieri)* and common carp *(Cyprinus carpio)* were stocked to a total number of 1,100 fish per mu.

The research period was from mid-April to mid-October. Details of stocking and harvesting are listed in Table 1.

Throughout the period, only fresh chicken manure was applied. No other organic matter was used. The amount of fertilization was limited to 3% (in dry weight) of fish body weight per day, spreading it evenly all over the pond, two or three times a week. The body length and weight were measured regularly to adjust the application.

The methods of collection and preparation of delta C samples were as follows:

Chicken manure: Fresh chicken manure was baked dry at 90-100°C for 10 hours prior to being fully ground; 2-5 g samples were randomly put into a test tube for measuring.

Chicken feeds: Samples of food pellets, corn flour, soybean cake and barley meal that we usually fed were collected and baked dry at 90-100°C for 10 hours before grinding for future use.

Bacteria: 100-ml samples of pond water from 8-10 points were collected and pump-filtered through a 20 μm mesh funnel to remove plankton. Under aseptic conditions, the filtered samples were transferred onto agar plates with chicken manure infusion as the only carbon source. The colony was scraped for future use after 48 hours' culture at 25°C. Experiment showed the carbon source was not influenced by the agar.

Organic detritus: About 100 ml of mid-water sediment samples were collected with a plankton net at different sites. The liquid part was removed after several hours' precipitating and the sediment was repeatedly rinsed by distilled water until no plankton was microscopically visible.

Plankton: 10-l samples of pond water from various locations were collected and filtered using 120-μm and 50-μm mesh filters and centrifuged for 10 min. at 4,000 and 5,000 rpm, respectively, for future use.

Attachments on rocks: Attachments on rocks just emerging from water upon drainage were scraped from 8 to 10 points along the pond side at the end of the experiment.

Pond bottom settlings: Settled matter on the sludge surface from 8 to 10 sites was collected with a home-made suction device. The liquid part was taken away after several hours' standing. The sediment was collected and blended with H_3PO_4 until the pH reached 1-2 with carbonate removed.

Sludge: Sludge out of 8-10 bottom sites was collected upon drainage and blended with H_3PO_4 until the pH reached 1-2 with carbonate removed.

Stocking fish: Three silver carp, bighead, baiji and common carp, respectively, were randomly taken from the fingerling rearing pond before stocking. Scales and epidermis were removed. Muscle tissues about 1 cm^2 and 0.5 cm thick were cut from the dorsal area and caudal peduncle. Blood, if any, was removed with distilled water. In addition, some dorsal fins and tail fins were cut for preparation.

Adult fish: At the end of the experiment, three fish of each of the four species were taken randomly to make samples as above.

Five to ten mg of each sample were put into a quartz bowl and burned at 850°C with an electrical oven in a lower vacuum system filled with oxygen. The gases produced, such as CO_2, flowed to a -50°C refrigeration piping to remove water vapor and then to a -196°C liquid nitrogen freezing separator to freeze the CO_2 while allowing surplus oxygen gas to escape. The CO_2 was then warmed to -50°C in a higher vacuum system and appeared as gas. The purified CO_2 in the collector was frozen to solid state at -196°C. Isotopic analysis of the pure CO_2 gas was done at room temperature on a MAT-250 type isotopic mass spectrometer.

Results and Discussion

Results of delta C sampling of fish body, natural food, chicken manure and feeds are presented in Tables 2-4.

The three tables show that delta C ranged from -13.83 to -28.76 ppt, while delta C of organisms in the pond was between -19.38 and -28.76 ppt. Carbon composition during the growth of all organisms can be distinctively identified with the delta C index.

Error of duplicate data randomly measured from some samples was within the range of ± 0.01-0.20 ppt. This is lower than the error of ± 1.0 ppt reported by Schroeder (1983b).

Table 2 shows that delta C in the fins of the four tested fingerlings was about 1 ppt less negative than that of fish flesh. The difference may be related to the carbonate content of the bones of the fin. This difference was also observed by Schroeder (1983b). Delta C in fish flesh at stocking was 4-5 ppt less negative than that of the fish at harvest. This indicates a difference in available feeds or feeding habit between this season and the previous season.

The four species were reared in the same pond before the experiment started. Their delta C index was -25.13 to -28.76 ppt. The main inputs to the pond were soybean juice, soybean residues and soybean cake (delta C typically -26 ppt). Unfortunately plankton delta C (the other main source of food carbon) was not measured during the pretest period. During the 180-day grow-out period, the added organic matter (manure) had a delta C of -18.2 ppt instead of -26 ppt of the soy. All the fishes gained weight several fold so that most of the carbon in their bodies at harvest was accumulated during the experimental period (see Table I). The difference in the delta C of carbon assimilated and accumulated by the fish this year as compared with the previous year indicates these changes in pond organic matter.

Organic fertilizers in manured ponds are a main source of carbon input. Green algae, which fix CO_2 in the atmosphere by utilizing sunlight, also provide carbon to fishponds. When manures are decomposed by a large quantity of aquatic bacteria and mineralized into nutritive salts, they can be used by phytoplankton for growth. Based on this, both the heterotrophic production system predominated by bacteria and autotrophic production system predominated by algae are formed through a series of autotrophic and heterotrophic production activities. The crisscross development and mututal promotion and interaction of these two systems form a complicated food web in the pond. The delta C values measured in the pond are summarized in Fig. 1.

The two water-column filter feeding species (silver carp and bighead) had delta C values of the pond plankton. "Settlings" likely represent a form of transient seston. The settlings were probably composed partly of precipitating dying phytoplankton and mainly of a "rain" of benthic matter that was temporarily suspended by the bottom feeding common carp and baiji. The relative abundance of the four seston fractions (plankton < 50 μm; plankton < 120 μm and 750 μm; plankton > 120 μm; settlings) was not measured. Schroeder (pers. comm.) has observed in other manured ponds stocked with pelagic filter feeders and bottom feeders, that the two most abundant forms of seston are phytoplankton < 50 μm and "settlings". In ponds with more than 150 filter feeders/mu (here there were 550/mu), settlings dominated the seston. The delta C of the two abundant forms of seston in our pond bracket the delta C of our filter-feeding fish. The delta C of these fish (slightly less negative than the settlings) indicates that the settlings were a significant source of nutrition. The delta C of a consumer is often 0 to 1 ppt less negative than its feeds (Schroeder 1983b).

Common carp and baiji are considered to be omnivores. Their delta C values were distinctly different from those of the pelagic filter feeders and indicate a dependence upon the benthic or sessile foods of the pond rather than seston. Unlike the distribution of foods in the seston, wherein it was possible to estimate their relative abundances, we do not have estimates of the relative abundances of the benthic foods. The bias of the delta C of the common carp and baiji toward the manure delta C indicates that the manure carbon was strongly present in their diets, either by direct consumption or via the pond's detrital food webs.

There were two dominant sources of carbon added to this pond: chicken manure (delta C -18) and microalgae (delta C -23). Using these data in a delta C balance (Schroeder 1983b), we can estimate the fraction of growth of the common carp and baiji which originated from each of these two inputs. We do not attempt a similar balance with the filter feeders because, as expected, their delta C values fall directly within the range of seston delta C values attributed to the algae. For such a case, the result of the delta C balance would be totally dependent upon which algal size fraction was dominant and we lacked direct measurements of this for this pond.

For the delta C balance of common carp:

X = fraction of carp carbon originating in the manure (delta C = -18)

(1-X) fraction of carp carbon originating in algae (delta C = -23)

$X(-18) + (1 - X)(-23) = -19.4$

$X = 0.72$; call it 70%

$(1-X) = 0.28$

or, if the muscle of the carp is 1 ppt less negative than the assimilated food:

$X(-18) + (1 - X)(-23) = -19.4 - 1$

$X = 0.52$, call it 50%

$(1-X) = 0.48$

Between 50% and 70% of the common carp flesh originated in food webs associated with the added manure. Conversely 30% to 50% originated with microalgae.

A similar balance for the baiji (delta C -20.9) shows 42% growth from manure-based food webs; if the baiji muscle delta C is 1 ppt less negative than that of the food, the result is 22% growth from manure carbon. Thus approximately 60% to 80% of the baiji growth originated from microalgal carbon.

This distribution of feeding patterns for CC and baiji is similar to that previously observed for common carp and tilapia in ponds receiving chicken manure.

Conclusions

In China, we are just beginning to apply the isotopic mass spectrographic analysis to fisheries research. Our preliminary delta C study implies that in a manured pond, the growth carbon of filter feeders (silver carp and bighead) is from the autotrophic production and suspended detrital systems. Approximately 2/3 of the growth carbon of one omnivorous species (baiji) is provided by autotrophic production system and 1/8 from the added manure carbon. Approximately 1/2 to 2/3 of the growth carbon of the other omnivorous species, common carp, comes from the manure food webs, and nearly 1/3 to 1/2 is from the autotrophic production system.

These facts have practical significance on guiding aquaculture activities toward developing pond management that will supply organic matter and mineral fertilizers at rates that encourage growth of useful natural foods. Exploration of pond dynamics is facilitated with this tool.

Acknowledgements

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Table 1. Stocking and harvesting.

Species	Stocking rate (no./mu)	Mean body weight prior to stocking (g)	Mean body weight in sampling (g)	Gain	Multiple of weight increase	Survival rate (%)	Harvest (kg/mu)	Net yield (kg/mu)
Silver carp	162	325	690	365	2.09	100	124.55	68.4
	362	50	340	290	6.80	100	122.40	106.0
Bighead	24	330	450	120	1.36	100	10.85	2.9
	82	50	220	170	4.40	100	18.20	13.9
Baiji	300	25	150	125	6.00	100	47.50	37.5
Common carp	150	10	280	270	28.00	86	36.43	40.5
Total								266.2 (22 kg/ha/day)

Table 2. Delta C in the pond fish.

Species	Samples	Delta C (ppt)
Silver carp		
stocking	fin	−26.41
	flesh	−27.63
harvest	flesh	−23.56
Bighead		
stocking	fin	−28.03
	flesh	−28.76
harvest	flesh	−24.26 −24.28 −24.20
Baiji		
stocking	fin	−25.85
	flesh	−26.42
harvest	flesh	−20.89
Common carp		
stocking	fin	−24.69
	flesh	−25.18
harvest	flesh	−19.43

Table 3. Delta C values in chicken manure and chicken feeds.

Samples	Delta C (ppt)
Chicken manure	−18.22
Chicken feed:	
Corn flour	−13.83
Soybean cake	−26.36
Barley meal	−21.32
Food pellets	−16.87, −16.68

Table 4. Delta C among the natural food and sludge.

Sample	Delta C (ppt)	Remarks
a. Plankton		
> 120 μg	−25.63 −25.73	Collected before the ending
> 50 and < 120 μg	−22.63	
< 50 μg	−23.02	
b. Organic detritus	−21.12	
c. Bacteria	−20.62	Gathered during the test and measured after culture
d. Attachments on rocks	−19.38	Sampled upon pond drainage
e. Precipitates		
unacidified	−25.55	Collected prior to stocking
acidified	−26.31	
f. Precipitates		
unacidified	−23.86	Sampled before the ending
acidified	−24.62 −24.42	
g. Sludge		
unacidified	−20.51	Sampled after the pond drainage
acidified	−23.13	

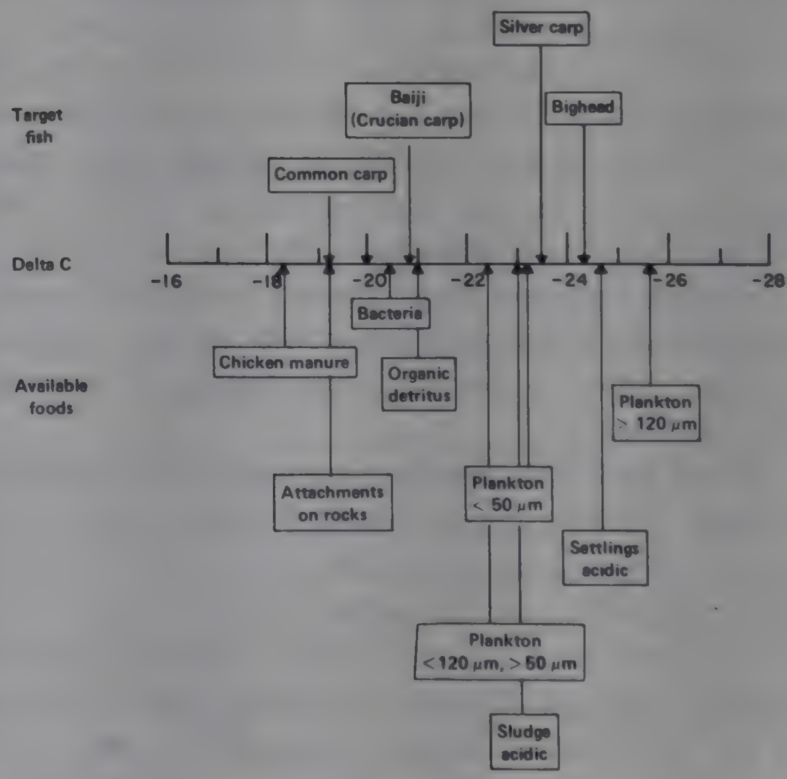

Fig. 1. Delta C values of available foods and the muscle of target fish at harvest.

The Chemical Quality of Waste Treated Waters and Its Relation with Patterns of Zooplankton Population

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KAPUR, K. and K.K. LAL. 1986. The chemical quality of waste treated waters and its relation with patterns of zooplankton population, p. 129-132. *In* J.L. Maclean, L.B. Dizon and L.V. Hosillos (eds.) The First Asian Fisheries Forum. Asian Fisheries Society, Manila, Philippines.

Abstract

Experiments were conducted on various chemical characteristics of waters treated with wastes from poultry, pigs, cows, goats and sheep individually as well as in combination in equal proportions, in addition to seven different combinations of the two most efficient (poultry-pig) wastes. Dissolved oxygen, BOD_5, nitrogen (ammonia and nitrates), phosphate, potassium and pH levels were analyzed. Changes in the levels of the parameters were correlated with the population patterns of *Scapholoberis kingi* raised separately on individual or combinations of wastes.

Introduction

Nursery ponds exhibit poor survival of young fish because of several factors, including lack of proper (mainly zooplankton) food. Some reports indicate that direct zooplankton production on the wastes is possible, bypassing the primary levels of production (Schroeder 1980; Kapur and Lal 1984). However, detailed investigations are necessary to study the feasibility of utilizing livestock wastes for the culture of commonly available species of zooplankton, in addition to working out their optimum requirement per fish for an effective nursery pond management.

These investigations summarize the results of findings on the relationship between the physico-chemical characteristics of waste-loaded waters and population buildup of the zooplankton, *Scapholoberis kingi*.

Materials and Methods

The waters were treated with safe level concentrations of various wastes worked out on the basis of static bioassay experiments (Kapur and Lal p. 133) and analyzed for dissolved oxygen (DO), BOD_5, ammonia, nitrate, phosphate and potassium levels after intervals of 3-30 days using the methods outlined in American Public Health Association et al. (1971). Simultaneously, changes in population density from an initial value of five per 500 ml were worked out.

Results

Data pertaining to experimental results are presented in Figs. 1-12. In all these cases, the peak zooplankton population coincided with or followed the maximum release of nutrients. As a result of poultry waste application, the zooplankton population increased steadily from an initial value of 5 to 75 adults and 275 juveniles within the first three days and attained a peak on the 15th day (3,450 adults, 12,150 juveniles) followed by a sudden decrease from the 18th day onward.

Figs. 1-5 show the pattern for the rate of mineralization: poultry > piggery > goat-sheep > cow dung, with a combination of all these wastes in between piggery and goat-sheep wastes. The maximum population buildup coincides with or follows the maximum availability/release of nutrients. The various combinations of poultry and piggery waste (Figs. 6-12) also exhibit a similar trend.

Discussion

Recently there has been greater emphasis on mass zooplankton culture (Nandy et al. 1977a, 1977b; Groenweg and Schluter 1981; Kapur and Lal 1984; Das, unpublished data; Nandy and Majumder, unpublished data).

The mass culture of *S. kingi* at safe doses of different livestock wastes indicates more or less a similar pattern of changes in population as well as in DO, BOD_5, nutrients and other characteristics, with all the wastes used. Zooplankton population rises steadily, with time, to a peak level (coinciding with the maximum release of nutrients with the least DO/highest BOD at safe levels), followed by a sudden fall to a medium level probably due to overcrowding, ensuing competition, lack of food and nutrients (Herbert 1978); then it stabilizes for a short time and subsequently falls.

The results of the relative efficiency based on maximum population level on a particular day follow the same order as exhibited by other parameters (Kapur and Lal, this vol.). However, determining the relative efficiency of different wastes and their combinations to raise the zooplankton crop have only been marginally attempted (Govind et al. 1978).

The main objective of nursery pond management is to raise a maximum number of crops per unit of area/time to fingerling stage. At least 15 days before stocking of nursery ponds in India, heavy manuring with cow dung at the rate of 10,000 kg/ha is used in order to provide a rich crop of zooplankton for young fry and simultaneously reduce the toxicity of the dung (Jhingran 1974). However, the time for manuring the pond and stocking fry depends on several factors including the fry and food availability. The zooplankton population attained a maximum level on day 15, 18, 24 and 21 upon subjecting the inoculum (10 adults/liter) to poultry, piggery, cow and goat-sheep wastes both individually and in combination in equal proportions.

The data obtained in this study can help to pinpoint the appropriate time of stocking. Table 1 recommends the prestocking waiting period for nursery ponds. Pond manuring with organic manure has long been practiced but the suitability of a particular waste, optimum dose for fry and zooplankton survival and growth, and the most appropriate stocking time have not yet been standardized. In the traditional manuring method, a waiting period before stocking is required which limits the number of crops that may be harvested. The present manuring rate may also be detrimental to certain zooplankton species (Kapur and Lal 1984, this vol.).

Simultaneous culture of both fry and zooplankton in a pond manured with safe levels of wastes may make possible the harvest of more crops within the same space and time, besides ensuring better survival of early fry due to enhanced food (mainly zooplankton). Such innovative approaches can solve the problem of quality fish seed shortage prevailing in the country.

The findings of this experiment conform with demonstrations of Schroeder (1980) and Kapur and Lal (1984, this vol.) that direct zooplankton cultures can be raised in dark laboratory conditions on organic wastes bypassing the primary productivity. At each trophic level in the food chain, there is a loss of energy (Odum 1971). The approach leads to greater energy economy by avoiding such losses (Odum 1971), besides ensuring better survival of the fish during its early stage by making available the protein-rich zooplankton food.

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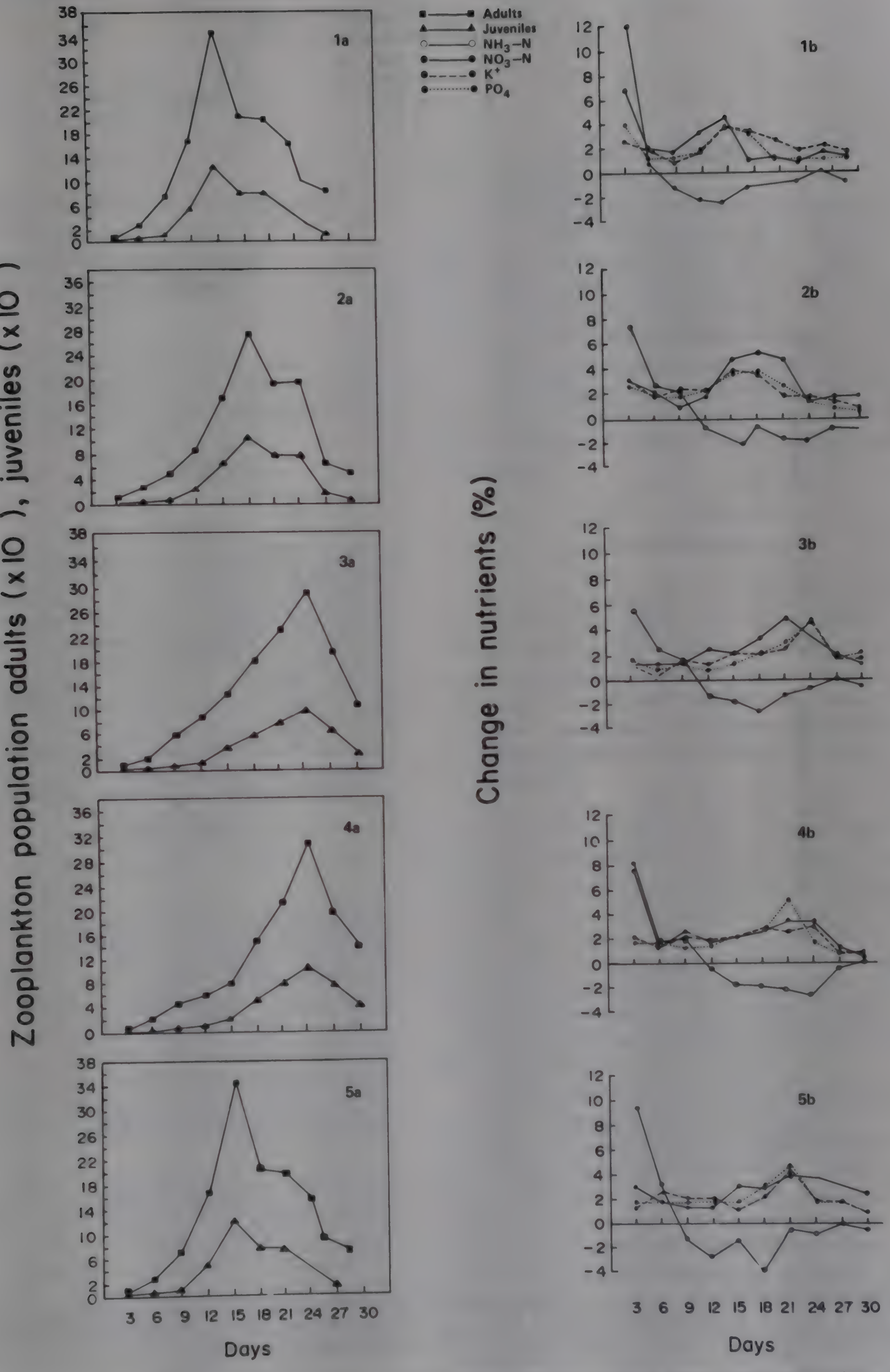
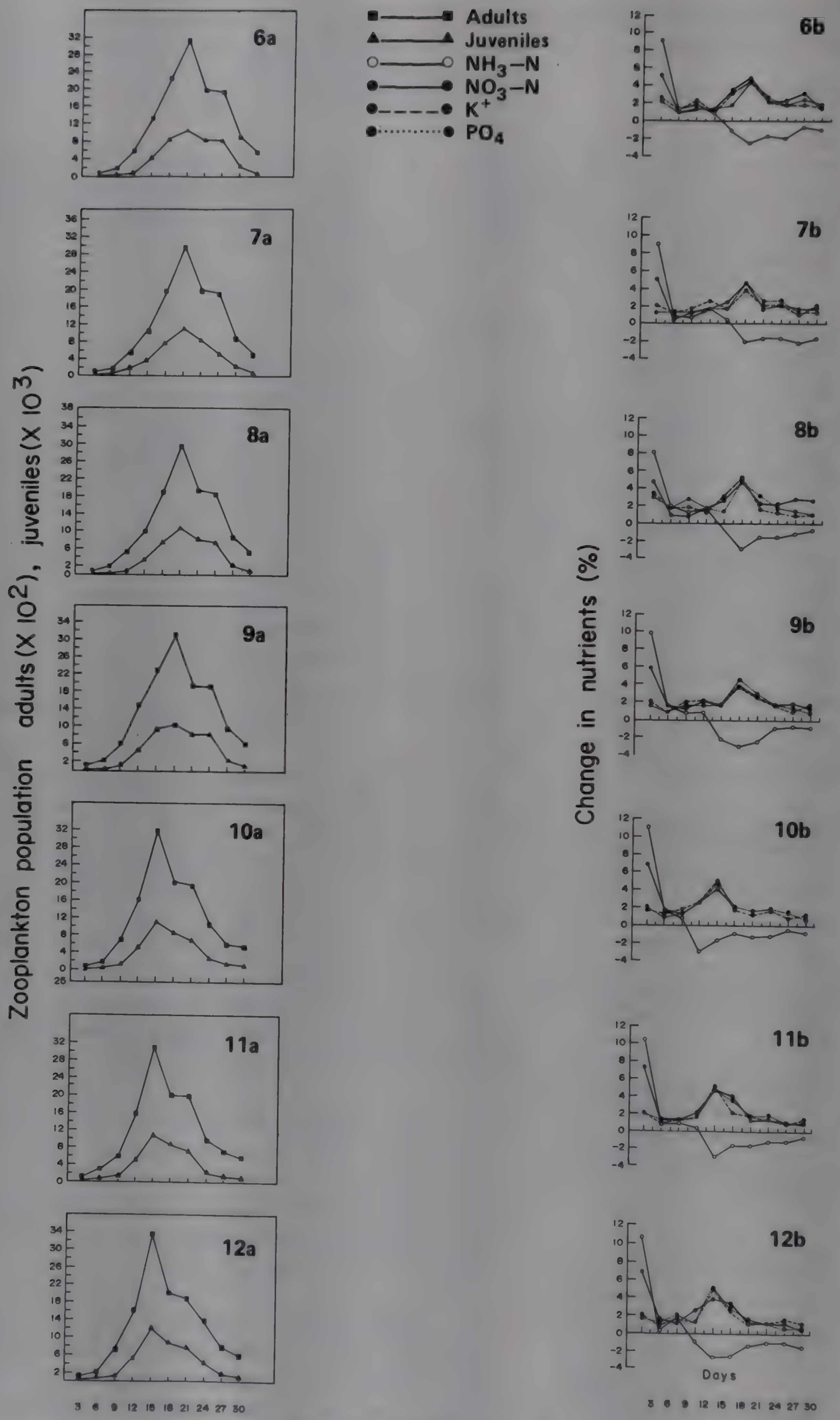
Table 1. Recommended prestocking waiting period for nursery ponds.

Type of waste	Prestocking waiting period (days)
Poultry	15
Piggery	18
Goat-sheep	24
Cow dung	24
Poultry:piggery:goat-sheep:cow dung 1:1:1:1	21
Poultry:piggery	
1:1	18
1:2	18
1:3	18
1:4	18
2:1	15
3:1	15
4:1	15



Zooplankton population pattern (a) in relation to changes in chemical characteristics occurring in waste loaded waters (b). Fig. 1. Poultry excreta. Fig. 2. Piggery excreta. Fig. 3. Cowdung. Fig. 4. Goat-sheep excreta. Fig. 5. Poultry:Piggery:Cowdung:Goat-sheep excreta 1:1:1:1.



Zooplankton population pattern (a) in relation to changes in chemical characteristics occurring in waste loaded waters (b). Fig. 6. Poultry: Piggery excreta 1:1. Fig. 7. Poultry:Piggery excreta 1:2. Fig. 8. Poultry:Piggery excreta 1:3. Fig. 9. Poultry:Piggery excreta 1:4. Fig. 10. Poultry:Piggery excreta 2:1. Fig. 11. Poultry:Piggery excreta 3:1. Fig. 12. Poultry:Piggery excreta 4:1.

Relative Toxicity of Certain Livestock Wastes for Fish Culture

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Abstract

Experiments were conducted to work out the relative toxicity of wastes obtained from poultry, piggery, cow and goat-sheep sheds individually as well as in combination in equal proportions and various poultry-piggery combinations. The parameters employed included BOD levels, LC_{50} values (24 and 48 hours), safe level concentrations and relative toxicity values of waste for zooplankton *(Simocephalus vetulus* and *Scapholeberis kingi)*, fry of *Cyprinus carpio* and hatching success of fertilized eggs. On the basis of these parameters the order of toxicity was: poultry (100), piggery (74.4), combination of four wastes in equal proportion (66.6), goat-sheep (58.4), and cow dung (51.2). The maximum loading levels of the wastes for fish culture systems in India were worked out from these data using cow dung as the base (10,000 kg/ha/year).

Introduction

Fish culture, developed along the western model, is highly energy intensive and not entirely suitable for developing countries such as India (Kapur 1984). At present, feeds utilized for fish culture in India alone constitute about 60% of the total input costs. Livestock wastes which form a significant alternative to costly feeds and fertilizers currently used in fish culture (Edwards 1980; Kapur 1981, 1984) contain about 71-79% N, 62-67% p and 82-92% K given in livestock feed (Taiganides 1978).

Fish in a culture system are known to improve the waste treatment capacity of a pond besides further increasing the food conversion efficiency of even pelletized food (Schroeder 1974, 1977; Kapur and Lal 1984). A properly manured pond thus develops a symbiotic relationship between the inhabitants and the medium in contrast to reduction in the fish growth and production and poor survival in improperly manured ponds (Schroeder 1980).

Cow dung is at present most widely-used in fish culture in India. Other wastes have not been tried effectively (Kapur 1981, 1984), partly because present knowledge is meager regarding: (1) their suitability and relative efficiency, both individually and in different combinations; (2) their maximum loading levels without affecting zooplankton and fish survival; and (3) their exact role in the trophic web in a composite fish culture system. The present investigations provide information on the relative efficiency of different wastes based on a number of parameters.

Materials and Methods

The poultry, piggery, cow dung and goat-sheep wastes for various experiments were obtained from livestock sheds of the College of Animal Sciences, Haryana Agricultural University, Hisar, India, and tried individually, as well as in suitable combinations. The wastes and waste treated waters were analyzed for their physicochemical characteristics following APHA (1971) and Suess (1982).

Test organisms for the experiments, namely, zooplankton *(Scapholeberis kingi, Simocephalus vetulus)* and fish *(Cyprinus carpio)* fry, were collected from the fish farm, Department of Zoology, and transferred to the Laboratory of Fish Biology and Endocrinology. Zooplankton were segregated and cultured in water medium using Baker's yeast at the rate of 1 ppm on alternate days. Carp fry were acclimated to laboratory conditions and provided with sufficient zooplankton and supplementary food in the form of rice bran:oil cake (2:3) at 1% body weight.

The test organisms were subjected to static bioassay procedure following APHA (1971), initially with broad dosages of 1-30% to determine the suitable concentrations for further detailed experiments. Suitable test concentrations from the above were investigated with respect to the mortality of treated organisms (initial number 10), being recorded every 24-120 hours. Three replications were made keeping an appropriate control. The regression of X (concentration of waste) upon Y (% mortality) was worked out (with X and Y plotted on logarithmic and linear scales, respectively) from which LC_{50} values were calculated.

For the studies on hatching success, fertilized eggs were obtained from a single pair (to minimize variation) of *Cyprinus carpio* in a breeding hapa in April to May 1985, with branched weeds as substratum for the attachment of eggs. Transparent eggs were segregated and collected along with a small portion of the substratum in wide-mouthed glass bottles and treated with dosages of different livestock wastes as above. Twenty "good" eggs (Kapur and Yadava 1982a) were exposed to various test concentrations and the LC_{50} values calculated as above.

Results

Data regarding the LC_{50} values, safe level concentrations and relative susceptibility values are presented in Tables 1-3. It may be seen very clearly from Table 1 that on the basis of 24 and 48 hours LC_{50} and safe level concentrations, the toxicity of the wastes to *S. vetulus* is in the following order: poultry > piggery > goat-sheep > cow dung. The combination of all the wastes in equal proportion exhibits a toxicity in between that of piggery and goat-sheep excreta. A comparison of the relative toxicity values calculated from the above indicates that toxicity of poultry waste is 2.73, 4.02, 4.34 and 2.89 times that of piggery, goat-sheep and cow dung, and combination of these four wastes in equal proportion, respectively.

From the above experiments, poultry and piggery wastes were marked as the two most toxic. The results of the experiment on combination of these wastes in different ratios demonstrate that the toxicity of the combination decreases in relation to increase in the proportion of piggery manure in the mixture.

The results of similar experiments on *S. kingi* (Table 2) indicate the same order of toxicity as above. However, the toxicity of poultry wastes is 1.62, 2.13, 2.36 and 1.72 times, respectively, that of piggery, goat, sheep and cow dung and combination of these wastes in equal ratio.

The experiments on *C. carpio* fry indicate a similar order of toxicity (Table 3). In this case, poultry waste is 2.56, 3.53, 4.07 and 2.70 times, respectively, more toxic than piggery, goat-sheep and cow dung, and combination of these wastes in equal proportion.

With regard to their effect on hatching success in common carp, the relative toxicity of different wastes and their combination in equal proportion exhibited an order of toxicity similar to that of experiments on zooplankton and carp fry (Table 4). The relative toxicity values indicate that poultry waste is 1.40, 1.62, 1.87 and 1.36 times more potent than piggery, goat-sheep, cow dung and combination of these wastes in equal proportion, respectively. Various poultry-piggery combinations were also tried and similar trends (Tables 1-3) were observed, with the combinations exhibiting toxicity in relation to the amount of poultry waste therein.

The data on BOD_5 and the percentage nitrate and phosphate released also followed broadly a similar order (Table 5). However, the percentage of nitrate released by equiproportion concentration of four wastes was next to that of poultry waste. Potassium release was maximum from piggery wastes.

Table 6 describes the relative efficiency of different wastes and their mean loading levels (with cow dung as base) based on data presented in Tables 1-5.

Discussion

Information on LC_{50} and safe levels of wastes seems to be previously lacking in the literature. However, this order is in conformity with the observations of Kapur (1981) on the effect of wastes on several parameters of productivity. The superiority of poultry and piggery wastes has been documented by several workers (Kapur 1981, 1984; Kapur and Lal 1984). However, according to Govind et. al. (1978) piggery wastes were inferior to cow dung.

These studies clearly demonstrate that the addition of piggery waste decreased the toxicity of the poultry waste in proportion to the amount of piggery waste in the mixture. The increase in fish production by about 20% as a result of using poultry-piggery wastes in 1:1 ratio in comparison to poultry waste alone (Kapur and Lal 1984) may possibly result from reduction in the toxicity of the mixture.

No report seems to be available on the effect of different livestock wastes on hatching success in any fish species, although the effect of a large number of environmental contaminants is well known (Kapur and Yadav 1982a, 1982b). Nevertheless, no morphological deformity was observed in the hatchlings obtained from waste-treated fertilized eggs.

The mortality of the test organisms at concentrations higher than the safe levels may partly be on account of high oxygen demand of the wastes, leading to lower dissolved oxygen levels in the medium, and due to some toxic principle such as ammonia (Kapur 1975). Moreover, the toxicity of most pollutants is enhanced by the reduction in oxygen level (Kapur and Toor 1978). Detailed study on the effect of pollutants related to oxygen levels/aeration is thus very essential.

Nursery pond management involves heavy fertilization with organic manures about 15-21 days prior to the release of fry, which may lead to mortality of certain zooplankton species. The determination of safe levels for fish fry and proper hatching will help in reducing the prestocking waiting period in common carp and increasing the number of fish seed crops obtainable per breeding season, while simultaneously stimulating the zooplankton population. Such innovative methods applicable under field conditions may help alleviate the problem of quality fish seed shortage. More work is required on other zooplankton and carp species.

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Table 1. Toxicity* of different livestock wastes individually and in certain combinations to zooplankton *S. vetulus.*

Type of waste	$LC_{50} \times gL^{-}$ 24 hr	48 hr	Safe concentration = (g/l)
Poultry	4.5 ± 0.93	3.5 ± 0.24	0.64
Piggery	37.0 ± 2.70	20.0 ± 2.35	1.75
Goat-sheep	16.0 ± 0.96	13.0 ± 1.31	2.57
Cow dung	41.0 ± 3.54	25.0 ± 1.41	2.78
Poultry:Piggery Cow dung:Goat-sheep 1:1:1:1	26.25 ± 2.31	16.2 ± 1.44	1.85
Poultry:Piggery			
1:1	6.1 ± 0.57	5.2 ± 0.57	1.10
2:1	5.9 ± 1.07	4.76 ± 0.49	0.93
3:1	5.3 ± 0.42	4.20 ± 0.24	0.85
4:1	5.0 ± 0.88	3.90 ± 0.59	0.71
1:2	6.4 ± 0.41	5.53 ± 0.27	1.24
1:3	7.4 ± 0.64	6.3 ± 0.91	1.37
1:4	8.6 ± 0.86	7.3 ± 0.82	1.58

*Mean ± S.E.

Table 2. Toxicity* of different livestock wastes individually and in certain combinations to zooplankton *S. kingi.*

Type of waste	LC_{50} (g/l) 24 hr	48 hr	Safe concentration (g/l)
Poultry	9.21 ± 0.68	7.30 ± 0.57	1.38
Piggery	15.27 ± 1.42	12.03 ± 0.40	2.24
Goat-sheep	26.69 ± 3.02	19.08 ± 2.21	2.93
Cow dung	23.23 ± 2.56	18.03 ± 0.83	3.26
Poultry:Piggery Cow dung:Goat-sheep 1:1:1:1	17.06 ± 2.16	13.19 ± 0.80	2.37
Poultry:Piggery			
1:1	13.11 ± 0.84	10.28 ± 1.08	1.90
2:1	12.38 ± 0.57	9.75 ± 0.51	1.81
3:1	12.47 ± 0.65	9.63 ± 0.80	1.72
4:1	10.64 ± 0.83	8.59 ± 0.74	1.68
1:2	13.26 ± 0.78	10.42 ± 0.79	1.93
1:3	14.28 ± 1.45	10.96 ± 0.76	1.94
1:4	14.56 ± 1.23	11.53 ± 1.54	2.17

*Mean ± S.E.

Table 3. Toxicity* of different livestock wastes individually and in certain combinations to *C. carpio.*

Type of waste	LC_{50} (g/l) 24 hr	48 hr	Safe concentration (g/l)
Poultry	3.5 ± 0.36	2.6 ± 0.43	0.43
Piggery	6.1 ± 1.28	5.1 ± 0.57	1.00
Goat-sheep	12.0 ± 0.93	9.0 ± 1.32	1.52
Cow dung	13.3 ± 1.04	10.1 ± 0.58	1.75
Poultry:Piggery Cow dung:Goat-sheep 1:1:1:1	11.5 ± 1.06	8.0 ± 0.87	1.16
Poultry:Piggery			
1:1	4.0 ± 0.36	3.2 ± 0.16	0.61
2:1	3.9 ± 0.29	3.0 ± 0.14	0.53
3:1	4.4 ± 0.43	3.2 ± 0.16	0.51
4:1	3.7 ± 0.36	2.75 ± 0.29	0.46
1:2	3.5 ± 0.14	3.20 ± 0.36	0.80
1:3	4.4 ± 0.57	3.8 ± 0.78	0.85
1:4	4.6 ± 0.43	4.1 ± 0.21	0.98

*Mean ± S.E.

Table 4. Toxicity* of different livestock wastes, their combination and poultry-piggery combinations to fertilized eggs of *C. carpio*.

Waste treatment	LC$_{50}$ (g/l)	Safe concentration* (g/l)
Poultry	0.87 ± 0.12	0.18
Piggery	1.22 ± 0.13	0.25
Cow dung	1.63 ± 0.09	0.34
Goat-sheep	1.41 ± 0.32	0.29
Poultry:Piggery:Cow dung: Goat-sheep 1:1:1:1	1.18 ± 0.17	0.24
Poultry:Piggery		
1:1	1.01 ± 0.13	0.22
2:1	0.96 ± 0.30	0.20
3:1	0.93 ± 0.12	0.19
4:1	0.91 ± 0.12	0.18
1:2	1.05 ± 0.15	0.21
1:3	1.07 ± 0.26	0.22
1:4	1.11 ± 0.07	0.21

*Mean ±S.E.

Table 5. The BOD$_5$ and nutrient characteristics of waste treated water.

Type of waste	BOD$_5$ (g/l)	Nutrient released* % age of total		
		Nitrate	Phosphate	Potassium
Poultry	19.35 ± 0.85	37.3 ± 4.67	55.8 ± 3.06	47.9 ± 5.83
Piggery	18.18 ± 1.63	34.1 ± 1.34	46.3 ± 4.99	54.4 ± 5.24
Goat-sheep	10.09 ± 1.25	33.2 ± 3.37	40.0 ± 2.86	41.7 ± 4.85
Cow dung	10.50 ± 2.15	27.4 ± 2.69	36.0 ± 2.15	36.8 ± 3.72
Poultry:Piggery: Cow dung:Goat-sheep: 1:1:1:1	12.10 ± 1.54	34.9 ± 2.0	44.1 ± 1.88	43.9 ± 2.78
Poultry:Piggery				
1:1	18.85 ± 0.25	Not determined		
2:1	19.10 ± 0.63			
3:1	19.15 ± 0.72			
4:1	19.15 ± 0.40			
1:2	18.60 ± 3.40			
1:3	18.50 ± 2.83			
1:4	17.28 ± 0.99			

*On 20th day at 35°C.

Table 6. Relative efficiency of different livestock wastes on the basis of safe levels for zooplankton, carp fry, fertilized eggs of *C. carpio*, BOD$_5$, and release of nutrients.

Type of waste	Safe levels				BOD$_5$ at 20°C	Maximum release of nutrients			Mean efficiency	Mean loading level* (kg/ha)
	S. ventulus	*S. kingi*	*C. carpio* fry	fertilized eggs of *C. carpio*		N	P	K		
Poultry	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	5,124
Piggery	36.57	61.61	39.09	72.72	93.95	94.83	85.09	113.42	74.41	6,886
Goat-sheep	24.90	47.01	28.29	61.74	54.26	92.19	71.72	86.96	58.38	8,777
Cow dung	23.02	42.33	24.57	52.87	52.12	73.54	64.55	76.88	51.24	10,000
Poultry:Piggery: Cow dung:Goat-Sheep 1:1:1:1	34.59	58.23	37.07	75.41	62.53	93.96	79.11	92.54	66.56	7,698
Poultry:Piggery										
1:1	58.18	72.63	70.49	83.64	97.42	Not determined			76.47	6,701
2:1	68.82	76.24	81.13	90.64	98.71				83.11	6,166
3:1	75.29	80.23	84.31	94.36	98.97				86.63	5,915
4:1	90.14	82.14	93.48	97.35	98.97				92.42	5,544
1:2	51.61	71.50	53.75	85.19	96.12				71.63	7,153
1:3	46.72	71.13	50.59	82.51	95.61				69.31	7,393
1:4	40.51	63.59	43.88	84.02	89.30				64.26	7,974

*Taking 10,000 kg/ha cow dung as basis.

An Integrated Semi-Intensive Shrimp and Livestock System in the Philippines

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Abstract

The development of a shrimp *(Penaeus monodon)* production system integrated with livestock was undertaken at the Sycip Plantation Inc., Negros Oriental, Philippines, as a result of crop diversification efforts in the province. Heavy organic manuring of brackishwater shrimp ponds strongly stimulated the production of *lablab* in the initial stages of pond preparation. Observations suggest that the problem of deteriorating water quality in the later stage of culture due to prawn burrowing/feeding activities, was exacerbated by constant addition of manures. Plankton analysis of pond water indicated that this was a result of excessive nanno- and picoplankton blooms. A highly significant correlation ($P < .001$) between plankton (cell size < 5 microns) count and BOD was obtained. An alternative system using "kitchen ponds" stocked with artemia provided a suitable source of food for shrimp growth. Daily additions of manure (100 kg dry weight/ha/day) produced an average 40 kg of mixed artemia and *lablab* protein each day during a three-month study period. Analysis of input:output ratios for the kitchen pond showed a net loss in organic matter and net gain in nitrogen yields during the onditioning period. Conditioned ponds had a net gain in both organic matter and nitrogen yields. An area ratio of 1:5 for kitchen pond to shrimp grow-out enabled production at 700-1,000 kg/ha/crop at 30,000 pieces/ha stocking density, 60-70% survival and grow-out period of 120 days. Significant reductions in feeding costs were obtained.

Introduction

The economic, social and ecological benefits accruing from integrating agricultural and aquacultural operations to enable recycling and efficient use of farm "wastes" have long been realized in freshwater aquaculture. However, this system of farming has not yet been commercially applied to brackishwater aquaculture.

Manure in both freshwater and brackishwater ponds generally improves yields of fish/shrimp (Moav et al. 1977; Schroeder 1978; Lee and Shleser 1984). Most work with the use of manures in ponds is with livestock adjacent to or over the culture ponds; manure passes directly into the ponds. Manure has also been used in the preparation of ponds for stocking or periodically added during culture.

Means of utilizing manure in integrated fish and shrimp pond production systems were investigated in this study to: a) provide an effective utilization of the manure directly or indirectly by the cultured species; b) prevent excessive water quality problems in culture ponds; and c) provide a sanitary and economical method of manure disposal.

Until the sugar market collapsed in 1982-1983, agriculture in the Negros Provinces of the Philippines was almost solely geared to sugar production. Farms have now been forced to diversify crop production. Large residues of crop byproducts and other materials for livestock feeds are being wasted in the region. Thus we conducted these studies on using byproducts in an integrated system by increasing our livestock populations and limiting them to a shrimp culture system.

The natural food of shrimp in the wild indicates an omnivorous diet of benthic plants and animals from a mainly detrital food web. In ponds, the feeding habits are not well documented and are controversial. Some farmers claim superior results using a benthic cyanobacterial mat of *lablab;* others prefer filamentous algae, such as *Cladophora* sp. and *Enteromorpha* sp. *(lumut)* and still others use aquatic halophytes like *Ruppia* sp. However, it seems that farmers' preferences rely on casual observations of gross morphology with little regard to the quality of these edible complex communities. Jumalon (1978) found wide variation in species composition, protein content and other definitive variables of *lablab* within a period of time in one culture pond.

In more intensive systems, supplemental feeding of trash fish/meat or pelletized formula feeds has been adopted although not always with reproducible results.

Indications of poor utilization of pellets in prawn ponds have led to the suggestion that disintegrating pellets may act as an organic fertilizer for a detrital food web on the pond benthos (Stanley and Moore 1983). Obviously, this is a rather expensive method of fertilization so it is essential to ensure that the pond bottom is rich before stocking, particularly in extensive and semi-intensive systems (< 1.5 t/ha/crop) in which there should be a strong dependence on natural productivity.

Effects of organic inputs on aquatic productivity are obvious in tropical estuarine areas where large amounts of mangrove leaves (Marten and Polovina 1981) undergo rapid degradation by cellulolitic decomposing microbes to form the basis of a detrital food web (Lee 1980). The utilization of manure in fishponds is a "carbon copy" of these natural food webs, modified to direct productivity towards a set goal.

However, there are disadvantages in adding manure during shrimp culture: (1) constant burrowing and benthic feeding activities of prawns lead to high turbidity such that settled manure is unavailable to phototrophic action; (2) shrimp cannot effectively utilize pico/nannoplankton, which predominate when manure is added during culture; (3) unsatisfactory water quality results in dissolved oxygen problems and development of epizootic infestations (e.g. *Zoothamnium* sp. and *Epistylis* sp.) on shrimp exoskeletons due to high bacterial populations (exacerbated by manure inputs) which are the principal food source of the peritrichous ciliates; (4) more frequent changes of water are required resulting in poor manure utilization.

The production of shrimp on a detrital-based food web is common in most culture systems; the use of livestock manures to augment this food web is a promising means of reducing costs in shrimp feeding.

The concept of a food production pond separate from culture ponds with high levels of organic inputs to stimulate productivity is not new (Anon. 1981). This production or kitchen pond (David, pers. comm.) enables farmers to use large inputs of manure without regard to shallow depth and excessive temperatures, which are favorable to *lablab*, and without dissolved oxygen problems associated with high levels of organic inputs. The "food" produced in these ponds is transferred to shrimp culture ponds as required. High yields (2 t/ha/day with 85% moisture) of good quality shrimp food, artemia-enriched *lablab*, can be harvested daily from the kitchen pond.

Materials and Methods

Various aspects of integrating livestock production with shrimp culture were studied from 1983 to 1986 at the Tamisu and C commercial farms of Sycip Plantation Inc. in Bais City and Manjuyod, Negros Oriental, Philippines. These include addition of manures in pond preparation and during shrimp rearing; effects of an integrated poultry flow-through fertilization system; linkage of cattle-fattening sheds to shrimp/fishpond systems and the use of liquid manures to support combined artemia-*lablab* kitchen ponds operated at high salinities (> 60 ppt).

Biological oxygen demand (BOD) was measured during various shrimp culture trials based on the Winkler titration method (Boyd 1979). Picoplankton (0.2-2.0 μm) and nannoplankton (2-5 μm) were analyzed from 500-ml water samples freshly collected in the middle of the pond and counted unfixed in a Neubauer hemocytometer using phase contrast microscopy. The environmental and biological characteristics of the flow-through fertilization system are given in Jumalon and Ogburn (in press).

Two kitchen ponds of 0.7 and 0.9 ha were monitored in terms of manure inputs and *lablab* harvest during the "conditioning" and "conditioned" period. Conditioning covered the first three months of kitchen pond operation, while conditioned period was one year after continuous operation. Total shrimp harvests and survival from 0.5-5.0 pieces/m^2 stocks were determined at the end of each culture period.

Results and Discussion

A strong exponential correlation ($P < .001$) between biological oxygen demand (BOD-12 hours) and the concentration of nanno- and picoplankton was found in the shrimp ponds over a one-year period ($R = 0.662$ and $n = 100$).

Counts of the pico- and nannoplankton groups during shrimp culture indicated that this size fraction of plankton became more numerous as shrimp biomass increased (Table 1) leading to the danger of low dissolved oxygen content of pond water in the early mornings.

The performance of the kitchen ponds is shown in Table 2. Yields of *lablab* equivalent to over 3.5 t/ha/mo were obtained at high manure loading during conditioning.

A net loss in the organic matter input:output ratio (1:0.85) during conditioning was probably due to accumulation of organic matter in the pond soil which increased from 4.5% to an average 12.3%. The input:output ratios of conditioned kitchen ponds, irrespective of artemia harvest, were 1:1.6 for organic matter and 1:2.9 for total nitrogen. Conditioned kitchen ponds yielded 92 kg/ha of organic matter and 25 kg/ha of *lablab* protein daily. Comparatively, this would represent the equivalent of 22 t/ha/year of soybeans, and indicates the prospects for protein production in brackishwater ponds utilizing liquid manures and blue-green algal pastures for protein synthesis. Increase in manuring rates can easily increase yields to 40 kg/ha of mixed *lablab*–artemia protein daily in conditioned ponds.

The breakdown of manure into *lablab* is very rapid: 4-10 hours depending on the type of manure and prevailing weather conditions, suggesting the presence of an active cellulolitic bacterial community.

The advantages in using an artemia-*lablab* kitchen pond are: good utilization of manure inputs; clearing of water column due to constant filtering activity of artemia, providing high light penetration to pond benthos; digestion and compaction of suspended bacteria, microalgae and manure particles by artemia, into sinking fecal pellets which concentrate organic residues on pond bottom; high salinity that ensures stability of production system as predators/competitors (e.g., cyanophages) are reduced and potential pathogens are eliminated; easy harvest of *lablab;* net increase in protein input:output ratio due to occurrence of certain species of the cyanobacteria which are heterocystous and known fixers of atmospheric nitrogen; use of liquid manure which is easier to handle in aquaculture operations and preserves nitrogen content (i.e., reduces NH_3 volatization); less danger of overmanuring as brine shrimp are hardy organisms that can tolerate adverse conditions such as low dissolved oxygen; and availability of a ready source of artemia for innoculation into newly prepared shrimp nurseries and grow-out ponds, in hatcheries and in diet formulations.

Preliminary results suggest an approximate food conversion ratio of about 150 kg fresh *lablab* from the artemia kitchen ponds to 1 kg of shrimp (FCR = 1:20 dry weight).

Good yields of large size shrimp (200-400 kg/ha/crop) have been obtained from an integrated system with a kitchen pond:grow-out ratio of 1:5. A combination of *lablab* and pellet (medium quality) feeding enabled higher stocking densities (3 pieces/m^2), increased yields significantly (700-1,000 kg/ha/crop with 70% survival in 130 days), improved feed conversion and reduced incidence of "softshelling" and nutrition-related diseases.

The system is somewhat labor intensive. In underdeveloped regions where cost of labor is low, unemployment high and poverty a problem, the social benefits of a high employment to capital ratio farming method such as this are evident. A number of modifications could be made to adapt the method to less labor-intensive systems.

With the vast areas of brackish/coastal water areas available throughout the world and with the current expansion of this promising method of food production, it is highly probable that integration will be practiced widely in future coastal pond development. Shrimp culture using this system of farming would enable significant reduction in feeding costs.

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Table 1. Plankton counts and secchi depth in manured and unmanured shrimp ponds and artemia kitchen ponds.

Culture period (weeks)	Shrimp pond <5 μm (cells/ml)	Shrimp pond Secchi (cm)	Shrimp + manure <5 μm (cells/ml)	Shrimp + manure Secchi (cm)	Shrimp + *lablab* <5 μm (cells/ml)	Shrimp + *lablab* Secchi (cm)	Artemia + manure <5 μm (cells/ml)	Artemia + manure Secchi (cm)
1	375,000	41	7,693,904	33	1,700,000	44	732,777	28
2	625,000	38	10,200,000	27	3,200,000	32	765,000	29
3	2,250,000	37	12,400,000	18	3,400,000	34	750,000	34
4	2,875,000	39	18,200,000	28	5,000,000	35	950,000	36
5	2,000,000	41	19,600,000	22	4,400,000	41	550,000	49
6	3,125,000	35	21,400,000	18	2,160,000	30	250,000	> 70
7	6,125,000	29	5,600,000	37	6,400,000	33	250,000	> 70
8	7,500,000	32	9,200,000	27	2,400,000	33	250,000	> 70
9	7,750,000	27	9,800,000	31	2,900,000	34	50,000	> 70
10	8,650,000	25	14,400,000	25	5,760,000	33	105,000	> 70
11	7,125,000	30	9,200,000	30	5,800,000	28	50,000	> 70
12	10,250,000	28	12,000,000	29	6,600,000	34	50,000	> 70
13	8,625,000	31	27,800,000	20	4,800,000	27	150,000	> 70
14	9,250,000	28	19,600,000	22	9,200,000	27	350,000	> 70
15	6,500,000	23	26,600,000	24	8,400,000	28	200,000	> 70

Table 2. Manure input and *lablab* output in artemia kitchen ponds.

Inputs and harvests	Conditioned Average (kg/ha/day)	Conditioning Kitchen pond 1 (kg/ha/month) Month 1	Month 2	Month 3	Period Kitchen pond 2 (kg/ha/month) Month 1	Month 2	Month 3
Manure input							
Dry weight	120.8	161.2	1,924.6	2,846.5	694.7	2,315.6	2,670.0
Organic matter	58.7	77.0	924.2	1,372.2	361.0	1,112.7	1,287.4
Nitrogen content	1.35	1.8	21.4	31.7	8.3	25.7	29.7
Lablab output							
Dry weight	308.0	409.0	2,403.0	3,083.0	796.0	2,108.0	3,571.0
Organic matter	92.4	91.0	534.7	686.0	232.1	614.7	1,041.3
Nitrogen content	3.9	3.6	20.9	26.8	10.3	27.2	46.1
Manure: *lablab* ratio							
Dry weight	0.40	0.39	0.80	0.92	0.87	1.10	0.75
Organic matter	0.63	0.85	1.73	2.00	1.81	1.81	1.24
Nitrogen content	0.35	0.50	1.02	1.18	0.95	0.95	0.65

Integrated Farming of Broiler Chickens with Fish and Shrimp in Brackishwater Ponds

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Abstract

An experiment on the integrated farming of broiler chickens with milkfish *(Chanos chanos)*, tilapia *(Oreochromis niloticus)* and shrimp *(Penaeus indicus)* in brackishwater ponds was conducted in 1,000-m^2 ponds of the SEAFDEC Research Station at Leganes, Iloilo, Philippines. Tested were varying densities of tilapia (500, 1,000, 1,500 and 2,000 for Treatments I-IV, respectively) with fixed densities of 5,000 shrimp and 200 milkfish per 1,000-m^2 pond. Poultry houses were constructed at the middle of each pond so that fresh chicken wastes would drop directly to the pond. Each unit was stocked with 90 broiler heads of three size groups, or a total of 180 heads for 120 pond culture days. Stocking, transfer and harvest of poultry were done every two weeks. Results indicated an average net production of shrimp of 19.15 to 28.38 kg/1,000 m^2; milkfish, 7.51 to 11.74 kg/1,000 m^2; tilapia, 33.68 to 66.97 kg/1,000 m^2 and chicken broilers, 180.90 to 217.39 kg/1,000 m^2. Statistical analysis revealed that the best net production of tilapia was obtained at a stocking density of 15,000/ha.

Introduction

In 1983, total world aquaculture production was 8.0 t x 10^6, of which 77.5% or 6.2 t x 10^6 came from the Asia-Pacific region (Csavas 1985). Brackishwater fishponds were the major and probably most reliable sources of fish supply. In 1980 the total area for aquaculture production in the South China Sea countries was 610,869 ha, of which some 66% or 404,777 ha were brackishwater areas. Of these, 80% were found in the Philippines and Indonesia (RAPA 1983). In a region where the average brackishwater fish farm holdings are less than 15 ha (Poernomo 1974; MNR-ADB 1983), where the very extensive practice of culture techniques yielded a mean production in 1980 of only 0.69 t/ha/year (RAPA 1983), the role of integrated farming becomes significant.

Integrated fish farming complements existing aquaculture practices such as the polyculture system. In the integration of chicken with fish farming, the ponds make use of chicken wastes and uneaten feedstuffs as fertilizers, thus increasing pond production of both fish and plankton through direct and indirect utilization of nutrients. Using the same amount of space, the farmer is provided with additional yields on easily marketable products like chicken, not to mention a source of protein for the farm family and the community.

This paper aims to evaluate the feasibility and profitability of integrated chicken broiler-fish/shrimp farming. A previous study on the integration of aquaculture with chicken farming used layer chickens, shrimp *(P. indicus)* and milkfish *(C. chanos)* (Apud and Pudadera 1983). It indicated that production can be increased further by increasing the stocking densities of shrimp and fish. As an offshoot, the present study introduces tilapia *(O. niloticus)* into the integration scheme aside from increasing the stocking densities of shrimp and chickens and maintaining the density of milkfish. Broilers were preferred over layers because broiler production can be programmed within a shorter pond culture period (three to four months). On the other hand, egg production of layer chickens can start only after six months of rearing.

Materials and Methods

Four treatments were tested based on the varying stocking rates of tilapia *(O. niloticus)* per 1,000-m^2 earthen ponds, i.e., 500 (treatment I), 1,000 (II), 1,500 (III) and 2,000 (IV). In addition, shrimps *(P. indicus)* and milkfish *(C. chanos)* were stocked at fixed densities of 5,000 and 200, respectively. Each treatment received direct chicken droppings from 90 broiler heads consisting of three different size groups. The treatments were replicated thrice on a completely randomized basis.

Poultry houses (4 x 8 m) were installed at the midportion of each pond. They were constructed from nipa and bamboo. Stocking, transfer and harvest of the broiler chickens were done every two weeks so that a total of seven harvests of broilers was realized. Supplemental feeding and inorganic fertilization were not given to the pond during the 120-day culture period.

The ponds were prepared by tilling the first 15-cm layer of the pond bottom to hasten oxidation of the soil. Drying and flushing of the pond bottom were done twice prior to the application of 100-kg lime ($CaCo_3$) per pond. Coconut fronds (10/1,000 m^2) were also distributed to serve as additional substrates for the shrimp fry.

Hatchery-produced shrimp fry (PL 15 with an average weight of 0.007 g) were stocked three days ahead of milkfish and tilapia fingerlings. Tilapia fingerlings (8.8 g average weight) were taken from a freshwater pond and acclimatized to 15 ppt prior to stocking. Pond water depth was maintained at a minimum of 70 cm. Water was replenished through tidal fluctuations.

The cages in each poultry house measured 1.5- m wide, 1-m high and 6-m long. Each cage was subdivided into three compartments to allow the stocking, transfer and harvest of chickens every two weeks. The 6-m long cage was apportioned into 1, 1 and 3 m to accommodate 30 heads each of day-old to two week old, two to four-week old and four to six-week old broilers. They were fed with a commercial chick booster mash (22% protein) from 1 to 10 days old and broiler starter mash (21% protein) from 11 days to harvest. Vitamin-mineral supplements and medications to prevent respiratory infection were also given. The chicks were immunized using the poultry viral preparation.

Milkfish were harvested first by seining as they swam against the incoming tidal water. The shrimps were harvested next by seining them when the pond water reached the pond bottom trench level. The remaining shrimps were handpicked after completely draining the ponds. After thorough washing, the harvests were placed in chilled water and classified according to size. They were placed in styrofoam boxes with crushed ice. A portion of the harvest was sold at the pond site and the rest were sold at the fish market.

The chickens were harvested upon reaching the minimum live weight of 1.2 kg. They were sold on a live weight basis.

Results and Discussion

Table 1 shows average net production of shrimp, milkfish, tilapia and chicken broiler using the integrated farming. The best net production of tilapia was obtained in Treatment III where the stocking density ws 15,000/ha. A further increase in stocking density (Treatment IV) drastically affected final weight and, consequently, net production.

Abrupt changes in the physicochemical parameters particularly salinity (Table 2), were experienced during the culture period due to floods brought about by two successive typhoons. No mass mortality occurred, demonstrating the hardiness and wide tolerance of shrimp, milkfish and tilapia to adverse weather conditions. They also showed minimal interspecific competition as indicated by insignificant differences in the total net production between treatments.

The gut content analysis showed that the chicken droppings were directly eaten by the shrimp, milkfish and tilapia. Results of pathological examination prior to harvest showed that they were not infected by any microorganism that might have rendered them unsuitable for human consumption.

Although the pond operation was able to profit in all treatments, Treatment IV, where the stocking density of tilapia was increased to 20,000/ha, produced the lowest net earnings as a result of higher inputs on tilapia fingerlings but lower net production and smaller harvest size. The poultry operations gave major contributions to the total net earnings. The occurrence of some chicken mortality in Treatment I due to the damaging effects of the typhoons on two poultry houses resulted in a deficit of net earnings. Based on the results obtained, profitability can be maximized by integrating the 90 chicken heads of three different sizes (total of 180 heads) with 10,000-15,000 tilapia, 50,000 shrimps and 2,000 milkfish/ha. Under these conditions, a return on investment of 16% can be realized (Table 3).

The raising of chickens is now a very well established technology as evidenced by the proliferation of several companies that manufacture high protein chicken feeds and, at the same time, supply their growers with chicks, medication and technical assistance. The integration of aquaculture with chicken farming provides several advantages, foremost of which is the assured supply of chicken manure which is one of the best organic fertilizers, in the growing of natural food in brackishwater ponds.

Chicken manure, like other organic wastes, can convert crude, inedible nutrient materials into high quality fish food. They can increase microbial activity in the water column and at the pond bottom by releasing the nutrients and minerals originally bound in relatively undigestible form. These in turn, provide the substrates for photosynthetic (autotrophic) and microbial (heterotrophic) production of basic fish food (Schroeder 1980). Fresh chicken manure contains 1.6% nitrogen, 1.5% phosphorous and 0.9% potassium (Woynarovich 1979). Furthermore, its total protein content is as high as 20-30%. About 80% of the manure represents undigested feedstuffs with 25% dry matter content. This is primarily due to the very short digestive tract of chickens; most of their excreta are only partly digested (Chen 1981).

In integrated chicken-fish farming the pond is continually fed in gradual amounts with chicken manure in contrast to the present practice of loading them only during pond preparation. In addition, more than 10-15% of the feeds given to the chicken can be used by the pond and its organisms due to their selective feeding and picking habits (Chen 1981). Lastly, using the same land space, manpower and equipment, additional income can be derived from chickens which are a highly valuable source of protein.

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Table 1. Growth, survival, and production of *P. indicus*, *C. chanos*. *O. niloticus* and chicken broilers over a 120-day culture period in 1,000 m^2 brackishwater earthen ponds.

		P. indicus				*C. chanos*				*O. niloticus*				
Treatments*	Pond no.	Initial BW (g)	Final BW (g)	Survival rate (%)	Net production (kg/pond)	Initial BW (g)	Final BW (g)	Survival rate (%)	Net production (kg/pond)	Initial BW (g)	Final BW (g)	Survival rate (%)	Net production (kg/pond)	Poultry production (kg)
I	4	0.007	7.11	59.5	21.12	2.56	80.81	51.0	7.73	8.8	81.88	83.2	29.70	168.50
	9	0.007	6.69	68.5	22.88	2.56	91.25	97.0	17.19	8.8	115.01	62.0	31.26	185.75
	12	0.007	7.01	71.6	25.04	2.56	228.25	21.0	9.07	8.8	158.35	56.2	40.09	188.44
	Mean	0.007	6.94	66.5	23.01	2.56	133.40	56.3	11.33	8.8	118.41	67.1	33.68	180.90
II	6	0.007	8.60	75.0	32.21	2.56	60.08	94.0	10.78	8.8	84.33	38.8	23.91	214.09
	2	0.007	9.52	74.3	35.31	2.56	88.14	79.5	13.50	8.8	106.86	41.4	35.43	214.63
	7	0.007	6.02	58.7	17.62	2.56	88.28	65.0	10.96	8.8	103.85	52.2	45.41	212.55
	Mean	0.007	8.05	69.3	28.38	2.56	78.83	79.5	11.74	8.8	98.35	44.1	34.91	213.76
III	11	0.007	8.26	60.5	24.93	2.56	92.46	49.0	8.55	8.8	103.48	63.1	84.69	205.84
	5	0.007	10.88	29.4	15.99	2.56	87.98	45.5	8.08	8.8	97.78	63.2	79.49	200.12
	3	0.007	6.73	49.3	16.53	2.56	121.08	26.5	5.90	8.8	71.76	46.4	36.74	214.98
	Mean	0.007	8.62	46.4	19.15	2.56	100.51	40.3	7.51	8.8	91.00	57.6	66.97	206.98
IV	10	0.007	8.56	67.1	28.68	2.56	115.35	64.5	14.37	8.8	69.07	45.0	44.56	207.01
	1	0.007	7.04	62.4	21.90	2.56	85.26	20.5	2.98	8.8	75.06	40.7	43.50	230.40
	8	0.007	6.59	73.5	24.23	2.56	82.14	36.0	5.48	8.8	66.35	47.1	44.90	214.76
	Mean	0.007	7.40	67.7	24.93	2.56	94.25	40.3	7.61	8.8	70.16	44.3	44.32	217.39

*Treatments I-IV had varying densities of *O. niloticus* (500, 1,000, 1,500 and 2,000/1,000 m^2 ponds, respectively) and fixed densities of 5,000 *P. indicus* and 200 *C. chanos* per 1,000 m^2 ponds. A total of 180 broiler heads per poultry house were reared for one culture period (120 days).

Table 2. Monthly averages and ranges of the physico-chemical parameters during the culture period.

Parameters	Time (6 AM 3 PM)	Treatments	Monthly average				Range
			August	September	October	November	
Dissolved oxygen (ppm)	AM	I	6.94	5.14	4.05	4.20	1.70 – 13.07
	PM		9.39	12.17	8.64	8.02	3.90 – 17.60
	AM	II	6.66	4.99	3.40	3.41	1.03 – 9.57
	PM		10.04	13.10	8.55	8.15	5.20 – 19.02
	AM	III	6.49	4.01	3.42	3.38	1.30 – 10.49
	PM		9.91	13.38	7.93	8.88	4.70 – 18.47
	AM	IV	6.68	4.59	3.37	3.78	1.50 – 9.93
	PM		9.58	13.86	9.48	8.37	5.80 – 19.07
Water temperature (°C)	AM	I	26.38	27.62	27.71	24.82	19.10 – 31.7
	PM		29.49	30.93	30.33	30.08	26.00 – 32.8
	AM	II	26.26	27.32	27.61	24.29	19.4 – 32.00
	PM		29.49	31.07	30.35	28.78	22.70 – 33.3
	AM	III	26.20	27.33	27.46	24.83	19.00 – 31.7
	PM		29.62	30.95	30.36	30.03	25.00 – 33.5
	AM	IV	26.18	27.46	27.68	24.33	19.3 – 31.80
	PM		29.52	31.07	30.35	30.08	26.3 – 32.37
Salinity (ppt)	AM	I	12.96	13.86	11.89	13.52	8.00 – 23.33
	PM		13.75	13.86	11.94	13.26	7.00 – 22.33
	AM	II	12.19	13.58	11.69	13.02	8.00 – 22.00
	PM		12.89	13.67	11.52	13.65	8.00 – 21.33
	AM	III	10.21	12.69	9.32	12.40	6.00 – 21.00
	PM		10.55	12.89	9.94	11.86	5.30 – 14.33
	AM	IV	12.02	13.60	11.53	13.82	8.00 – 23.67
	PM		12.81	13.89	11.89	13.97	8.30 – 23.67
pH	AM	I	7.39	7.33	7.42	7.68	6.29 – 8.80
	AM	II	7.86	8.11	8.23	8.04	6.99 – 8.90
	AM	III	7.39	7.22	7.46	7.30	4.90 – 8.79
	AM	IV	7.45	7.32	7.36	7.39	4.80 – 8.57

Table 3. Input-output analysis of integrated chicken broiler-fish-shrimp farming in 1,000 m^2 earthen ponds for 120 days culture period. (US$1.00 = ₱18.00)

Items	Treatments (refer to Table 1)			
	I	II	III	IV
I. Production (Sales)				
A. Pond				
1. *P. indicus*	920.00	1,120.00	760.00	1,000.00
2. *C. chanos*	240.00	240.00	160.00	160.00
3. *O. niloticus*	456.00	552.00	960.00	744.00
Subtotal	1,616.00	1,912.00	1,880.00	1,904.00
B. Poultry				
1. Chicken broilers	5,430.00	6,420.00	6,642.00	6,540.00
Total	7,046.00	8,332.00	8,522.00	8,444.00
II. Capital cost				
A. Pond development	208.33	208.33	208.33	208.33
B. Poultry house	638.83	638.83	638.83.	638.83
Total	847.16	847.16	847.16	847.16
III. Running cost				
A. Pond				
1. Shrimp fry	450.00	450.00	450.00	450.00
2. Milkfish fingerlings	60.00	60.00	60.00	60.00
3. Tilapia fingerlings	150.00	300.00	450.00	600.00
4. Lime	80.00	80.00	80.00	80.00
5. Labor	375.00	375.00	375.00	375.00
6. Miscellaneous	113.36	113.36	113.36	113.36
Subtotal	1,228.36	1,378.36	1,528.36	1,678.36
B. Poultry				
1. Day-old broiler chicks	1,170.00	1,170.00	1,170.00	1,170.00
2. Poultry feeds	2,481.00	2,481.00	2,481.00	2,481.00
3. Feed supplements and medications	245.66	245.66	245.66	245.66
4. Labor	562.50	562.50	562.50	562.50
5. Electricity	323.40	323.40	323.40	323.40
6. Miscellaneous	170.05	170.05	170.05	170.05
Subtotal	4,952.61	4,952.61	4,952.61	4,952.61
Total	6,180.97	6,330.97	6,480.97	6,630.97
IV. Total cost (II and III)	7,028.13	7,178.13	7,364.13	7,478.13
V. Gross earnings	7,046.00	8,332.00	8,522.00	8,444.00
VI. Net earnings				
A. Pond	179.31	325.31	143.31	17.31
B. Poultry	(161.44)	828.56	1,050.56	948.56
Total	17.87	1,153.87	1,193.87	966.87
VII. Return on Investment (VI/IV)	0.25	16.07	16.21	12.91

Fecal Coliforms as Index of Pollution in an Integrated Pig-Fish Farm System

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Abstract

Two fishponds measuring 20 x 50 m stocked with 2,000 fish consisting of 85% *Oreochromis niloticus*, 10% *Cyprinus carpio* and 5% *Channa striata* were used to determine the level of fecal coliforms in the pond water and fish flesh. One pond received fresh pig manure at 40 kg/week; the other, inorganic fertilizer at 5 kg/week. Water samples were collected at weekly intervals for a period of eight weeks from three sites, namely, at a point closest to the manure dumping site, at the middle of the pond and farthest from the manure dumping site. Fish samples of 60 tilapia and 18 carps were collected monthly for a three-month period. Both water and fish samples were analyzed for coliforms with the multiple-tube fermentation technique. Results showed coliform counts with mean concentrations varying from 2,874.30/100 ml of water in the middle of the pond to 1,810.70 farthest from the manure dumping site. Differences in concentration between collection sites were not significant ($P > 0.5$) but mean values obtained weekly differed significantly ($P < 0.05$). Tilapia had coliform counts of 124.5 while carps had 76.5-158.0 coliform/100 g flesh. Differences among fish samples of the same species were not significant ($P > 0.05$) but were significant between species ($P < 0.05$). Biochemical tests revealed the presence of *Escherichia coli* and *Klebsiella* sp. as indicators of fecal pollution.

Introduction

In recent years, the fish production drive in the Philippines has been geared towards development of measures to accelerate freshwater aquaculture production. Experiments have demonstrated that considerable fish production can be obtained when ponds are fertilized. Increases in the cost of commercial fertilizers, however, inhibited their use and called for other sources such as animal manures. Although fishpond fertilization with manure (pond manuring) in other Asian countries has a very long history, it has never been tried extensively in the Philippines. The waste organics serve to fertilize the pond directly, causing algal blooms and also serving as food for bacteria in the pond. In Singapore, China, Thailand and Taiwan, pig manure is used to fertilize fish farms (de Guzman and Chia 1978; Woynarovich 1979; Buck et al. 1979). In Hongkong and the Philippines, 100 pigs weighing 30 kg each are considered adequate to fertilize and maximize total revenue and profit of a 1-ha fishpond (Bardach et al. 1972; Hopkins et al. 1980).

Traditional fish farming in Asia has recycled animal manures for a long time regardless of hazards associated with it. As pond manuring causes organic enrichment, it also hastens the deterioration of water quality. Thus, the aquatic environment becomes favorable for the growth, sustenance and multiplication of pathogenic bacteria that are hazardous to fish consumers. More important is that coliforms are introduced into the pond aerobic and facultatively anaerobic gram-negative, nonsporeforming rod-shaped bacteria that ferment lactose with gas formation within 48 hours at 35°C (APHA et al. 1975). The enteric bacilli included in the coliform group are *Escherichia coli, Klebsiella, Enterobacter* (formerly *Aerobacter)* and several genera previously classified as "paracolon" organisms, namely, *Serrtia, Edwardsiella* and *Citrobacter* (Davis et al. 1973). The occurrence of fecal coliforms in fish is a reflection of the pollution level of their water environment because coliforms are not the normal flora of fish (Rudolfs et al. 1950; Cuelin 1962; Rao et al. 1968; Cohen and Shuval 1973; Evison and James 1973). It is believed that the contaminations of fish tissues are mostly results of surface or intestinal contamination (Salle 1964; Banwart 1979) and fish may carry those infections if caught in polluted waters (van Duijn 1973; Caldreich and Ciarke 1966).

The purpose of this study was to determine the level of coliforms in an integrated pig-fish farming system.

Materials and Methods

Two fishponds of the Central Luzon State University (CLSU) Freshwater Aquaculture Center Nueva Ecija, Philippines, with areas of 1,000 m^2 (20 x 50 m) each were made available for the study. The ponds were stocked with 2,000 fish consisting of 85% tilapia *(Oreochromis niloticus)*, 10% carp *(Cyprinus carpio)* and 5% snakehead *(Channa striata)*. One pond received fresh pig manure at 40 kg/week from a herd of 14 pigs and the other pond with

5 kg inorganic fertilizer (N-P-K, 16-20-0)/week. Water samples for bacteriological analysis were collected in sterile BOD bottles weekly for eight weeks from three pond sites, namely, at a point closest to the manure dumping site (3 m from the pig pen), at the middle of the pond (25 m from the pig pen) and at a point farthest from the manure dumping site (45 m from the pig pen). Fish were randomly sampled by seining the pond over a three-month period. Total samples added up to 60 tilapia and 18 carps. Due to difficulty in retrieval, no snakeheads were obtained. Ten grams of flesh was dissected from the proximal upper quadrants on the lateral side of each fish, ground in a sterile blender and diluted with an equal volume of diluent.

The multiple tube fermentation technique of determination for members of the coliform group was used as adapted from APHA (1975). The procedure is divided into three parts, namely, presumptive test, confirmed test and completed test. A total of three tubes per dilution using 1 ml, 0.1 and 0.01 ml portions were inoculated from each sample of water and fish flesh suspensions. The formation of gas in the inverted Durham tubes constituted a positive reaction for both presumptive and confirmed tests while formation of gas in tubes and demonstration of gram-negative, nonsporeforming, rod-shaped bacteria were considered satisfactorily completed tests. To differentiate coliforms of fecal origin from others of nonfecal sources, the EC test was performed. Gas formation in the fermentation tubes within 24 hours or less was considered a positive reaction. Tests for biochemical reaction of the coliform organisms were also conducted.

The number of positive findings of coliform group of organisms (either presumptive, confirmed or completed) resulting from a multiple portion decimal-dilution plating was recorded as the Most Probable Number (MPN) of coliforms in the water and fish flesh suspensions.

Results and Discussion

The mean number of coliforms obtained in the water from the three collection sites of the manured pond over an eight-week period is presented in Table 1. Coliform counts obtained from the point closest the manure dump increased from the first to the fourth weeks, declined on the fifth and sixth picked up on the seventh and declined again on the eight week. The pattern was similar to that of coliform counts obtained at the middle of the pond.

Water samples obtained farthest from the manure dump had variable data; the first week had a low value which increased during the third week, decreased on the fourth and fifth weeks decreased drastically on the sixth week and steadily increased during the seventh and eighth weeks. Highest coliform count was in the middle of the pond followed by the point closest to the manure dump and lastly, farthest from the manure dump (Table 1). Differences between the coliform counts of the three collection sites were not significant ($P > 0.05$) but differences in mean values obtained weekly were significant ($P < 0.05$).

The coliform counts obtained from the three sites at different periods of sampling fluctuated markedly. This can be attributed to certain biological phenomena occurring in the manured pond. The pig manure dumped in the pond served as fertilizer which enhanced algal bloom. These algae served as food for plankton which, in turn, were eaten by the fish. The moment algal bloom was enhanced, the dissolved oxygen possibly decreased and carbon dioxide in the water and biochemical oxygen demand increased. Low concentrations of dissolved oxygen (0.3-0.4 mg/l) in the pond water favored the survival of fecal and nonfecal coliforms.

Die-off rate of coliforms in wastewaters is due in part to the high algal demand for carbon dioxide (Danes et al. 1964; Chay 1975). Moreover, the design of the pond which sloped towards the middle, favored the concentration of the fish in that area. When manure was dumped, however, the school of fish moved to the dump, then returned to the middle of the pond, creating some current in the water that dispersed the manure particles as well.

No coliforms of fecal origin were isolated from fish from the inorganically fertilized pond. Table 2 shows the number of coliforms obtained from tilapia and carps in the manured pond over an eight-week collection period. Values for both fish were not significantly different ($p < 0.05$) over time but levels were significantly different between the two species. The organisms reached the fish flesh by their gills or skin but mainly through the intestines (Banwart 1979; Salle 1964).

Based on biochemical tests, two species of coliforms, namely, *Escherichia coli* and *Klebsiella* sp. were isolated from the water samples and from tilapia and carp flesh. Table 3 shows the number of fish samples positive for organisms. In both species, about half had *E. coli* and the majority had *Klebsiella* sp.

Growth of *E. coli* has been observed with as little as 0.28 ppm organic matter in solution (Allen et al. 1952). Waste material was much more abundant in the present experiment. *E. coli* is used as indicator of fecal pollution of water. *Klebsiella* sp., on the other hand, is normally a bowel organism. It is referred to as the "colon bacillus" and its presence in the water supply usually indicates fecal contamination. (Andre et al. 1967; Davis et al. 1973; Pelchar et al. 1977; Chay 1975).

The present findings suggest that measures should be adopted to sterilize the pig manure to kill the microorganisms prior to loading it in an integrated pond. Fish harvested from such a system should be thoroughly cleaned and properly cooked.

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Table 1. Coliform count of water samples (MPN/100 ml water).

Collection site*	Frequency of collection (week) 1	2	3	4	5	6	7	8	Mean
A	364.40	600.00	2,611.10	3,133.30	977.70^{a}	$2,565.50^{b}$	$5,111.10^{c}$	4,122.20	$2,484.40^{a}$
B	616.70	1,555.60	$1,900.00^{a}$	$2,955.60^{b}$	$4,400.00^{c}$	$5,111.10^{d}$	3,133.30	4,122.20	$2,974.30^{a}$
C	257.80	1,538.90	1,555.60	700.00^{a}	$1,688.90^{b}$	500.00^{c}	$2,677.80^{d}$	5,566.70	$1,810.70^{a}$

a, b, c, d = Mean values within rows with different superscripts are statistically significant ($P < 0.05$).

*A (closest to manure dump), B (middle of the pond), C (farthest from manure dump).

Table 2. Coliform of fish (MPN/100 g flesh).

Month of collection	Fish species Tilapia	Carps
September	176.0^{a}	88.0^{b}
October	209.5^{a}	158.0^{b}
November	124.5^{a}	76.5^{b}

Mean values with different superscripts are significantly different ($P < 0.05$).

Table 3. Species of coliforms.

Fish	Total samples	*E. coli* No.	%	*Klebsiella* sp. No.	%
Tilapia	60	27	45	56	93.33
Carp	18	9	50	15	83.33

Comparative Economics of Rice-Fish Culture and Rice Monoculture in Ubon Province, Northeast Thailand

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Abstract

The economics of rice-fish culture and rice monoculture were investigated by field studies of six farms in the Lam Dom Noi irrigated area of Ubon, Thailand. On each farm, inputs to and production of rice fields stocked with fish and of unstocked fields were monitored by weekly visits to the participating farmers between June 1984 and June 1985. Profits/ha/man-day/farm were compared for the 1984 rainy season, the 1985 dry season, and the full 12-month period. Fish species used were *Cyprinus carpio, Oreochromis niloticus (x mossambicus?)*, and *Puntius gonionotus*, stocked at 2,500-5,000/ha. By the end of 1984, the value of production had not yet recovered initial investments and operating costs of the rainy season. Fish harvests were not yet significant, but there was consistent evidence of higher rice yields from fields stocked with fish. During the following dry season, production of both table and seed fish became more significant. There was further evidence of the positive effect of fish culture on rice yields, especially when fish were stocked at high densities. The dry season profit/ha/man-day compared favorably with that from rice monoculture. After one year, the value of rice-fish culture production had exceeded investments, but the year-end profit was still less than the one-year profit from rice monoculture. Self-sufficiency in seed fish by farmers could probably reverse this situation. This study indicates that the practice has potentials for raising the standard of living of low-income rice farmers in northeast Thailand. Its applicability in rainfed situations is also considered.

Introduction

The rice farmers of northeast Thailand are one of the poorest groups in the country. Confronted by severe financial and environmental constraints, they try to improve their situation by maximizing the efficient use of their meager resources rather than by increasing inputs to unaffordable high levels. They are normally not inclined to risk already scarce resources on unfamiliar new practices.

The Thai Department of Agriculture, through the Farming Systems Research Institute, has, therefore, been investigating rice-fish culture as one farming practice that could possibly improve the circumstances of farmers in northeast Thailand. Rice cultivation normally requires flooding, so it is logical to consider the introduction of fish with low-cost dietary requirements into this rice-based system.

The field modified for rice-fish culture normally requires few changes. It must hold water long enough to assure adequate fish production. The dikes around the field must be high enough to withstand serious flooding. The presence of a small pond or trench at least 50 m deeper than the surrounding field is advisable as a refuge for the fish in times of low water. The non-revolutionary nature of rice-fish culture and the affordable investment it requires make it a low-risk practice which the Ubon farmers could readily try.

This study investigated the comparative economics of rice-fish culture and rice monoculture over two consecutive seasons on six farms in the Lam Dom Noi irrigated area of Ubon Province, northeast Thailand. The area lies very close to the hills of Laos in a minor rain shadow. The soil, while highly variable, is generally poor both in fertility and water-holding capacity. The irrigation system normally functions during the dry season, roughly from mid-December to mid-May, and at other times, in case of emergency.

Materials and Methods

Participating farmers were selected in May 1984 and during the subsequent month they prepared their fields to receive fish which were stocked in June. Fields on three farms received 2,500 fish/ha; the other three were stocked at 5,000/ha. Of the fish stocked, 20% were common carp *(Cyprinus carpio)*, while the remaining 80% was equally composed of Tilapia *(Oreochromis niloticus (x mossambicus?))* and tawes *(Puntius gonionotus)*.

Each farmer was left to manage his system as he saw fit. Project staff gave suggestions when advisable, and interviewed farmers weekly to monitor inputs, practices, problems, and sales of fish and rice.

Units of input and output were measured or weighed when possible, but the emphasis was on monitoring rather than direct measuring. Farmers cannot normally wait for researchers before applying inputs or harvesting; thus, some degree of imprecision was inevitable. Farmers, however, can give reasonably accurate descriptions of their activities and estimates of costs and expenses. Using these, and measuring samples occasionally, enabled the researcher to obtain and express units of input and production in commonly understandable terms.

This same methodology was used for the dry season activities (January-June 1985). The only modification was that fish densities were not controllable because of natural breeding by tilapia and carp. However, stocking rates and other data could still be monitored.

Results and Discussion

Consistent evidence of a positive effect of rice-fish culture on rice production emerged from 1984 rainy and 1985 dry season crops (Tables 1 and 2).

Before these data were subjected to analysis of variance, rice yields/ha were averaged for all fields with fish and without fish in each farm for the 1984 rainy season. Following this, each set of data was analyzed separately by farm. In neither case was rice production significantly different in fish fields from that in unstocked fields nor from projected production. It is felt that a larger sample would have indicated a significant difference between actual and projected production.

The results reported in Table 3 (fish production) have been pooled. Production from individual farms ranged from 37 to 273 kg/ha. The overall range of production is expected to decrease and the overall average to increase as farmers learn from their experiences and from one another. Biological production, furthermore, is considerably higher than this because all farmers had an important amount of their fish left in the fields at the end of the reporting period. This could not be estimated with any degree of precision. During the period of the report, the value of fish sales per family ranged from 70 to 1,237 baht.

In terms of individual growth potential, common carp seems to do better than the other two species stocked. Results from the 1985 dry season and ongoing studies indicate that this generalization applies when carp are stocked at densities equivalent to, or somewhat higher than those of the other two species.

In terms of fry and fingerling production, tilapia, not surprisingly, has the greatest potential; however, natural reproduction by common carp was significant in January, following the December opening of the irrigation system. One of the six farmers reported the presence of two to three hundred *Puntius* fingerlings in late May from a field with an abundant supply of natural food.

As with the fish production, variation among farmers was obscured by the pooling of the economic data (Table 4). However, the overall picture which emerges is felt to be a valid one.

Profit was defined as the total value of rice and fish production minus all monetary inputs incurred. Inputs not paid for were considered time inputs.

All operations had reached the break-even point in rice-fish culture after one year. Overall profitability/ha/man-day had not yet surpassed that of rice monoculture by the end of the report period. This situation is expected to change for a number of reasons. First, two of the three farmers considered for the rainy season and annual economic evaluation hired help to dig trenches and heighten dikes. This amounted to 14% of the total annual financial outlay for the three. This will not be a recurring investment, and normally, the farmers do not hire help but would use family labor or its equivalent. Second, another 39% of the year-end financial input reported here is made up by fingerling costs. This is the most essential initial investment cost in rice-fish culture. A farmer self-sufficient in seed fish can save considerable money and normally assure the profitability of his operation. With a little care, self-sufficiency in tilapia and common carp should not be difficult for most irrigated farmers. Third, fish production in the second and subsequent years should increase over that of the first year. The farmer's managerial skills should improve with experience, and the standing crop of fish available at the beginning of the subsequent rainy season should normally be greater than that of the first year.

The low-risk nature of rice-fish farming makes it attractive to the target group. The modifications to traditional rice farming and the small investments it demands can be accommodated by the farmers. The low level of pesticide used in northeast Thai rice fields further reduces the risks involved in the practice.

Several benefits are becoming evident from rice-fish culture. At this point, however, only speculative reasons can be given for the apparent positive effect of rice-fish culture on rice yields. Farmers tend to pay more attention to fields stocked with fish and often add supplementary feed. Much of this feed will eventually become organic fertilizer. By grazing, fish are likely to convert pest organisms to fish flesh and fertilizer. Weeds, especially, are rare in fields stocked with appreciable numbers of fish. Some farmers report that rice production is distributed more evenly in fields stocked with fish. Certainly fish moving through a rice field would distribute their wastes more evenly than normally would a farmer adding manure. The digging action of common carp may further aid rice production by oxygenating the soil.

So far, only circumstantial evidence exists for a positive effect of fish on soil fertility. Considering the points just made, the organic content of the soil and the rate of nutrient recycling should increase due to fish action. This is important in the poor soils of much of northeast Thailand. In partial support of this, the most dramatic apparent improvements in rice production to date have occurred in fields with the poorest soils and/or those stocked with high densities of fish.

Farmers have encountered a few problems in adopting the practice. Thefts occur rarely. Newly-transplanted rice can be uprooted by large fish and *Puntius* can jump from the water to eat ripe rice grains. Digging by common carp can undercut banks. Some farmers feel that the introduced species, notably carp and tilapia, drive off some of the larger native fish species. Fry stocked into a poorly prepared nursery pond or rice field risk facing massive mortalities, and farmers should aim to minimize predators and maximize naturally available feed before stocking.

Despite these problems, most member farmers are expanding their operations and many of their neighbors are adopting the practice.

This paper has considered rice-fish culture in an irrigated situation. Most northeast Thai farmers are rainfed. To what extent does this study apply to them?

Two major points are in contrast. The second fish-growing season enjoyed by irrigated farmers cannot be expected in rainfed areas. Fish production will therefore normally be limited to that period when the field can hold an adequate amount of rainwater. Returns on initial investments would therefore accumulate more slowly than from irrigated farms.

Keeping a year-round supply of water for holding parent fish can be more difficult for a rainfed farmer. This will make self-sufficiency in fingerlings more difficult. Solutions to this problem, such as shallow drilled wells and tanks, have already been suggested. They deserve testing.

Table 1. Rainy season rice production (kg/ha) from fields with and without fish on four farms, Ubon, Thailand, 1984.

Field	Farm			
no.	A	B	C	D
1	1,028 (fish)	678 (fish	4,496 (fish)	2,365 (fish)
2	531 (without)	626 (without)	4,640 (fish)	1,709 (without)
3	485 (without)		3,991 (fish)	
4	573 (without)		3,395 (without)	
5			2,462 (without)	

Table 2. Projected and actual rice yields (kg/ha) for fields first stocked in 1985.

	Season		
Field No.	Rainy 1984	Projected dry 1985	Actual dry 1985
A-1[a]	1,088	N/A[a]	1,100
A-2	588	594	1,025
A-3	538	544	556
A-4	606	613	738
C-3[a]	3,988	N/A[a]	1,575
C-4	3,394	1,338	1,525
C-5	2,463	975	1,644

[a] Reference fields, stocked with fish in 1984. Dry season production was not expected to change, relative to reference fields, between seasons.

Table 3. Fish production in rice fields by season and species, Ubon, Thailand.

		Puntius			Common carp			Tilapia			
Season and use	Area (ha)	No.	Av. wt. (g)	Total wt. (kg)	No.	Av. wt. (g)	Total wt. (kg)	No.	Av. wt. (g)	Total wt. (kg)	Total (kg)
Rainy 1984											
Table	1.466	332	54	17.8	83	147	12.17	223	62	13.82	43.79
Dry 1985											
Table	1.635	142	90	12.78	938	108	101.5	349	69	24.1	142.95
Dry 1985											
Seed	1.635	–			629	19	17.6	1,731	7	12.1	29.65
Both: Table	N/A	475	64	30.6	1,021	111	113.7	572	66	37.9	186.7
Seed	N/A	–			629	19	17.6	1,731	7	12.1	29.65

Season and use	Production per hectare (kg)	Weight fish sold (kg)	Income (Baht)
Rainy 1984			
Table	29.9	23.7	640
Dry 1985[a]			
Table	87.43	85.04	2,438
Dry 1985			
Seed	18.13	27.44	1,357
Both: Table	117.3	108.7	3,078
Seed	18.13	27.44	1,357

Notes: Only table fish were produced in 1984.
Seed fish production does not include those kept by the farmer.

[a] Includes some fish not counted, and therefore not included in individual species totals.

Table 4. Seasonal and annual economic balance sheets for rice-fish culture and rice-only cultivation, Ubon, Thailand.

	Rice-fish culture			Rice-only cultivation		
Practice season	Rainy[a]	Dry[b]	Both[a]	Rainy[a]	Dry[b]	Both[a]
Area (ha)	0.825	1.64	N/A	1.746	2.21	N/A
Production value (baht)	5,175	7,507	10,669	5,381	4,037	8,609
Inputs (baht)	6,024	2,061	7,246	965	2,329	2,598
Profit (baht)	849	5,446	3,443	4,416	1,708	6,011
Profit per hectare (baht)	1,029	3,330	3,020	2,529	774	3,997
Time input (man-days)	134	124	222	99	113	154
Profit per man-day (baht)	6.33	43.92	15.44	44.61	15.12	39.08

[a] Totals for three farms only.
[b] Totals for six farms.

An Economic Analysis of Coastal Shrimp Culture in a Mixed Farming System, Chittagong-Cox's Bazar Region, Bangladesh

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Abstract

Using linear programming and parametric resource programming as analytical tools, a sample farm survey of two selected villages in the Chittagong-Cox's Bazar coastal region, Bangladesh, showed that with traditional methods of shrimp farming there is little possibility of increasing farm income. However, a shift to the improved technology in the culture of shrimp yields a higher net revenue for the farm even with a smaller farm size. With improved methods of shrimp culture, an increase in the availability of capital (through the supply of institutional credit) could remove restrictions toward the expansion of lands for shrimp culture within the farm. It would also result in an increase in the farm demand for labor. Therefore, the introduction of improved shrimp culture techniques in the coastal area would have two main implications. Firstly, it would facilitate the release of lands for use by other farms without affecting farm income. Secondly, support of institutional credit would enable the existing farms to expand. In both cases, the net effect would be an improvement in the farm income and employment opportunities in coastal villages.

Introduction

As a farm enterprise, shrimp culture in the coastal brackishwater areas of Bangladesh plays an important role in generating income and employment for the coastal farming people. However, the technology is traditional and extensive with a low yield, of 30-50 kg/ha (Kibria 1985). Therefore, it is important to introduce yield-augmenting technology in such a way that it can easily be incorporated by the coastal area farmers, where a farm plan consists of a mix of shrimp and other production activities.

This paper is based on an investigation (Ahmed, unpublished data) of the status of shrimp culture in the farm production planning of the coastal area farmers. The feasibility of incorporating improved methods of shrimp culture in a mixed farming framework were studied, as were the capital constraints on the use of improved technology. Lastly, the effects of variations in the availability of institutional credit on optimal production decision were examined.

Materials and Methods

The study used the linear programming technique for optimal analysis of an average farm. Two models were computed to represent both traditional and improved technology situations in the culture of shrimp by setting up linear programming problems for a given initial year. The objective was to maximize farm net revenue subject to various resource and production constraints. The results of the optimal solution can be utilized to evaluate the status of shrimp farming in farm production plan. Also, a comparison of the two models shows how a shift to an improved technology optimal plan would affect farm income and employment situation. Parametric linear programming was used for analyzing the effects of variations in the availability of institutional credit to finance farm working capital. The parametric variations of institutional credit were done for the model that reflected the improved technology situation. The data were mainly from sample surveys of two selected villages in the Chittagong-Cox's Bazar coastal region. Additional data were provided by secondary sources (e.g., Bangladesh Bureau of Statistics).

Results

As shown in Table 1, net revenue is higher although the farm size is lower in the improved technology optimal plan compared to the traditional technology optimal plan. However, the two plans utilize the same amount of working capital, Tk42,100 (30 Taka = US$1), which is also the maximum amount available to the farm.

Further, a comparison of the optimal plan results for the existing (traditional) technology model and the practice by the farmers in the study area (Table 1) shows that the farm size in the optimal plan is 59.10% higher than the average farm size (6.21 ha) found in the survey information. But the net revenue in the optimal plan (Tk114,550) is only 8.81% higher than the average net revenue for the farms under study. Thus, net revenue/ha of farm size is Tk11,607 in the optimal plan, whereas for the farmers under actual operation it is Tk16,953. However, the requirement of working capital is much lower in the optimal plan than the average amount required by the farmers in actual operations. As shown in Table 1, working capital requirement/cropped ha in the optimal plan (Tk3,942) is about 34% lower than the average requirement by the farmers. Moreover, the optimal plan gives a higher ratio of net revenue to total working capital; 272% as against 178% for the farmers' actual operations. Thus, in the optimal plan, land productivity is lower (net revenue/ha), while in practice capital productivity (net revenue per unit of capital) is lower.

The recommendations provided by the optimal plan cannot bring about any substantial change in the existing farm income with the use of existing traditional methods of shrimp farming. Rather a reorganization of the farm plan through improved technologies (e.g., semi-intensive shrimp culture) might be able to generate the same net revenue with much less expenses than that at present, or be able to generate a much higher net revenue with the same amount of capital applied under the present pattern.

Given the available farm resources - the amounts of various types of lands, labor and capital - as well as the choices in the selection of different enterprises, shrimp cultivation occupies the largest area of the total farm lands under both the existing technology and improved technology optimal plans. Table 2 shows that the shrimp enterprise occupies more than 80% of the total cropped area in both the optimal plans.

Table 2 also shows the proportions of total net return from different enterprises, that shrimp contribute 80.5% and 87% of the total farm net revenue in the existing and improved technology optimal plans, respectively. The proportion of working capital as well as that of labor required by different enterprises are shown in Table 3. Shrimp cultivation demands most of the working capital (76% in the existing technology optimal plan and 92% in the improved technology optimal plan) and 60% or more of total labor required for the whole farm plan.

The improved technology model offers more choices of activities and productive practices in shrimp culture (e.g., semi-intensive culture). On the other hand, the traditional technology model includes only the existing crop and shrimp practices. A comparison of the results of the two models show the effects of introducing improved shrimp culture technique, given the existing resource base, on the cropping pattern, cropping intensity, farm income (net revenue) and factor use intensity.

Table 4 shows that the introduction of semi-intensive techniques results in more intensive and diversified farm practices. However, total farm size has been reduced to 4.42 ha from 9.87 ha (55.22% decrease). Further, the intensity of cropping defined as the ratio of total cropped area to total operating holdings is higher (196%) in the improved technology optimal plan compared to that in the existing technology optimal plan (108%).

Table 4 also shows that farm net revenue increases from Tk114,557 to Tk126,638 (an increase of 10.52%) if the improved technology optimal plan is followed. Moreover, the use of improved technology is able to generate a higher net revenue/cropped ha as well as per holding ha. The net revenue/cropped ha increases from Tk10,727 to Tk14,589 (36% increase), while net revenue/holding ha increases from Tk11,606 to Tk28,267 (146.46% increase).

The effect on labor demand and, hence, on farm employment varies. As shown in Table 5, moving from the traditional technology plan to the improved technology plan, the total farm demand for labor decreases from 1,027 man-days to 786 man-days (a decrease of 23.46%). There is a slight increase in family labor (0.25%), but the demand for hired labor decreases by 66.88%. As such, the proportion of hired labor to total labor is reduced from 35.34% to 15.29%. The reason for the fall in the total labor demand is the decrease in farm size (by 55.22%), and also to changes in the diversification of farm activities.

The intensity of use of the major production factors, e.g., land, labor and capital, in farm operations depends to a large extent on the cropping pattern and the technology.

The indicator of land use intensity is given by the measures of the cropping intensity index. One such measure is the crop intensity index (CII), a time weighted land-use index that evaluates the fraction of the total hectare months available to the farmer for production with a given pattern of cropping (Paris et al. 1982). The CII is 100 if the lands under production are used in all 12 months. Table 6 shows the intensity of land use as provided by CII increases from 61.0% to 96.50% when a shift occurs from the existing technology plan to the improved technology one. Therefore, introduction of new technology in shrimp farming will enable more crops to be raised and at the same time, allow a more intensive use of land.

Secondly, labor use indicator is the total man-days/ha for all activities during the planning period (Yang 1965). Labor demand/ha of farm holding will increase because of more intensive (multiple cropping) practices. As shown in Table 6 labor absorption/ha of farm holding increases from 104 man-days in the existing technology optimal plan to 178 man-days in the improved technology optimal plan (71.15% increase). However, demand for labor/ha of cropped area shows slight decrease (5.65%). This is because a shift to the improved technology optimal plan causes a change in the selection of activity mix that is relatively less labor intensive. For instance, selection of semi-intensive shrimp culture replaces salt production that requires more labor/ha.

Finally, the capital use indicator employed here is the total cost of variable inputs/ha, which is the same as the working capital requirement/ha for all enterprises during the planning period (Yang 1965). In this regard, although the two optimal plans use the same amount of total working capital (Tk42,100), the intensity of capital use per ha is much higher in the improved technology plan compared to the existing technology optimal plan. This is because farm size in the former is smaller than that in the latter. Table 6 shows that capital requirement/ha of farm holding increases from Tk4,265 in the traditional technology optimal plan to Tk9,525 in the improved technology optimal plan (123% increase).

For the improved technology model, the availability of working capital in terms of parameters depended on fluctuations in the supply of institutional credit. Solutions were obtained from a level of zero credit supply to Tk103,320 from the institutional source.

As shown in Table 7, the effects of increased supply of capital (credit) on optimal farm income is positive. There is a large scope for increasing farm net revenues through increasing farm size (by leasing additional wet lands), as well as cropping intensity from the level of resource constraints specified in the improved technology model, provided that increased provision of the cheaper institutional credit is made available to farmers (Ahmed, unpublished data).

The effects of credit parametrization on total labor demand by the farm are also positive. The successive optimal plans with higher and higher amounts of available institutional credit absorb a higher amount of both family labor and hired labor. The absorption of family labor increases from 92 to 99.7% when credit supply from institutional source reaches Tk81,459 after being increased from zero (Table 8). The requirement of hired labor increases throughout the range of credit parametrization.

On the other hand, labor requirement/ha decreases with successive increments of credit. However, net revenue/man-day of labor increases from Tk119 to Tk202 when credit supply increases from zero to Tk103,320. It should be noted at this point that even though labor absorption/ha decreases with increasing supply of capital (Table 8), total demand for labor will eventually increase via increase in farm size. As shown previously in Table 6, under the existing traditional technology, the optimal plan using 9.87 ha of land absorbs only 104 man-days of labor/ha, whereas with a level of credit supply amounting to Tk103,320 the farm plan using about the same amount of land (9.53 ha) is able to absorb about 136.67 man-days of labor/ha (Table 8).

Conclusions and Policy Implications

A comparison of the results of the improved technology model and the traditional technology model shows that the introduction of improved methods of shrimp culture could bring higher net revenue per unit of farm land. However, given the existing resource base, this will reduce the total farm size, implying that it will facilitate release of lands for use by other farmers without affecting farm income. Also, the total demand for farm labor, specifically hired labor, will decrease. To the individual farmer this means a reduction in his dependence on hired labor. Nevertheless, the results of credit parametrization show that availability of adequate capital (institutional credit) will enable the expansion of farm lands under shrimp culture and, at the same time, allow adoption of more improved technologies. Expansion will increase total demand for labor. Therefore, introduction of new technology in shrimp culture, supported by institutional credit, will not only increase income for the farm, but will also create employment opportunities in the coastal area.

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Table 1. Existing farm practice[a] and optimal farm plan results

Items	Existing practice	Optimal plan results Traditional (existing) technology	Improved technology
Size of farm (ha)	6.21	9.87	4.42
Cropping intensity (%)	159.00	108.00	196.00
Total cost (excluding cost of fixed assets) (Tk)	58,692.00	42,100.00	42,100.00
Net revenue (Tk)	108,278.00	114,567.00	128,840.00
Working capital requirement/ha cropped area (Tk)	5,948.00	3,942.00	4,860.00
Ratio of net revenue to total working capital (%)	178.00	272.00	301.00

[a] Figures under "Existing Practice" are averages for all respondent farms.

Table 2. Total cropped area and farm net returns for each enterprise in the optimal plans

	Proportion of total cropped area		Proportion of total net return[a]	
Enterprise	Improved technology	Traditional technology	Improved technology	Traditional technology
Rice	7.60	11.33	6.08	9.84
Shrimp	86.42	81.09	87.17	80.52
Salt	2.96	7.58	3.21	9.64
Vegetables	2.96	0	1.85	0
Betel-leaf	0.06	0	1.69	0
Total	100.00	100.00	100.00	100.00

[a] The net returns for each enterprise is the difference between the gross-revenue and total variable cost, which does not include cost of hired resources (e.g., hired labor, borrowed capital and hired land in this case).

Table 3. Proportion of total working capital (variable cost) and labor required by each enterprise in the optimal plans.

	Proportion of total[a] working capital		Proportion of total labor	
Enterprise	Improved technology	Traditional technology	Improved technology	Traditional technology
Rice	4.60	15.99	9.41	11.49
Shrimp	92.09	76.10	61.58	59.30
Salt	1.22	7.91	12.08	29.21
Vegetables	1.56	0	8.87	0
Betel-leaf	0.5	0	8.14	0
Total	100.00	100.00	100.00	100.00

[a] Excluding cost of hired resources (hired labor, interest on borrowed capital and rent of hired land).

Table 4. Cropping pattern, cropping intensity and net revenue in optimal plans with traditional and improved technology.

Items	Traditional technology	Improved technology
Net revenue (Tk)	114,557.00	126,638.00
Total size of farm (ha)	9.87	4.42
Cropping pattern (ha)		
1. Betel-leaf[a]	0	0.007
2. Vegetables[b]	0	0.256
3. Rice	1.21	0.458
Single crop	1.21	0
Single crop in rotation with vegetables	0	0.256
Double crop	0	0.202
4. Salt	0.810	0.257
Single crop	0	0
Single crop in rotation with shrimp	0.81	0.257
5. Shrimp	8.66	3.96
Single crop (traditional)	7.76	0.15
Single crop (traditional) in rotation with salt	0.81	0.257
Single crop (intermediate)	0.09	0
Double crop (semi-intensive)	0	0
Multiple cropping intensity (%)	108.00	196.00
Net revenue/holding/ha (Tk)	11,607.00	28,651.00
Net revenue/cropped/ha (Tk)	10,727.00	14,589.00

[a] Betel-leaf enterprise is assumed to be a single-crop activity covering the whole year.

[b] The vegetable enterprise is a single crop activity in rotation with rice.

Table 5. Labor demand under different optimal plans.

	Traditional technology (man days)			Improved technology (man days)		
Monthly	Family	Hired	Total	Family	Hired	Total
January	60.00	0	60.00	58.54	0	58.54
February	60.00	0	60.00	60.00	70.53	130.53
March	60.00	197.76	257.76	60.00	46.13	106.13
April	54.60	0	54.60	53.92	0	53.92
May	26.78	0	26.78	35.70	0	35.70
June	60.00	22.48	82.48	50.27	0	50.27
July	60.00	0	60.00	60.00	0	60.00
August	60.00	110.66	170.66	60.00	0	60.00
September	42.00	0	42.00	48.97	0	48.97
October	60.00	32.25	92.25	60.00	4.76	64.76
November	60.00	0	60.00	60.00	0	60.00
December	60.00	0	60.00	58.54	0	58.54
Total	663.38	363.15	1,026.53	665.94	120.24	786.36

Table 6. Factor use intensity under different optimal plans.

Items	Traditional technology	Improved technology
Crop intensity index (CII) (%)	61.00	96.50
Total labor holding hectarage (man days)	104.00	178.00
Total labor cropped hectarage (man days)	96.00	91.00
Total working capital holding hectarage (Tk)	4,265.00	9,525.00
Total working capital cropped hectarage (Tk)	3,942.00	4,850.00

Table 7. Farm credit variations and optimal farm net revenues.

Amount of institutional credit (Tk)[a]	Net revenue (000 Tk)	Farm size (ha)	Net revenue/ha (000 Tk)	Net revenue/unit of working capital
0	87.50	3.46	25.300	3.95
4,508	96.60	3.46	27.92	3.63
5,282	98.16	3.46	28.37	3.58
8,027	103.69	3.46	29.97	3.44
8,263	104.16	3.46	30.10	3.43
9,710	107.07	3.46	30.95	3.38
20,000	126.60	4.42	28.64	3.00
25,322	136.77	4.91	27.86	2.88
29,964	145.57	5.11	28.49	2.79
34,582	154.32	5.48	28.16	2.72
35,340	155.71	5.54	28.11	2.71
40,170	164.48	5.93	27.74	2.64
40,269	164.66	5.91	27.86	2.64
40,460	164.99	5.94	27.78	2.63
41,220	166.32	5.98	27.810	2.60
51,265	183.63	6.67	27.53	2.50
51,328	183.73	6.68	27.50	2.50
60,452	198.90	7.28	27.32	2.41
81,120	233.20	8.66	26.93	2.26
81,459	233.74	8.66	26.99	2.26
95,087	255.75	9.53	26.84	2.18
95,882	256.89	9.53	26.96	2.17
97,220	258.11	9.53	27.08	2.20
103,320	264.35	9.53	27.74	2.22

[a] This is over and beyond the amount available from other sources of capital; price of institutional credit assumed to be 25%/annum.

Table 8. Labor requirement and net revenue per man day in the optimal plan at different levels of institutional credit.

Amount of institutional credit (Tk)	Hired labor (man days)	Family labor (man days)	Total labor (man days)	Labor requirement/ha (man day)	Net revenue/man day (Taka)
0	72.14 (09.82)[a]	662.56	734.70	212.25	119.10
4,508	76.73 (10.30)	668.38	745.11	215.26	129.84
5,282	77.51 (10.37)	669.40	746.91	215.76	131.42
8,027	80.31 (10.66)	672.95	753.26	217.61	137.85
8,263	81.33 (10.77)	673.78	755.11	218.15	137.90
9,710	87.23 (11.41)	677.53	764.76	220.94	140.00
20,000	120.25 (15.20)	665.96	786.21	177.84	161.00
25,332	137.00 (17.23)	657.96	794.96	161.74	172.00
29,964	156.00 (18.89)	669.84	825.84	161.49	176.00
34,582	183.40 (21.13)	684.68	868.08	158.48	177.80
35,340	188.38 (21.54)	686.02	874.40	157.88	178.00
40,170	221.91 (24.28)	691.96	913.88	154.18	180.00
40,269	215.90 (23.75)	689.32	905.22	153.14	181.90
40,460	218.35 (24.04)	689.87	908.22	153.02	181.86
41,220	221.79 (24.34)	689.25	911.04	152.35	182.25
51,265	289.01 (29.13)	703.11	992.12	148.77	185.00
51,328	292.26 (29.32)	704.64	996.90	149.14	184.30
60,452	354.15 (33.00)	709.35	1,063.50	146.00	187.00
81,120	499.11 (41.20)	712.74	1,211.85	140.00	192.43
81,459	503.29 (41.21)	717.86	1,221.15	141.09	191.40
95,087	604.56 (45.76)	716.50	1,321.06	138.54	193.59
95,882	604.26 (46.08)	707.73	1,311.99	137.63	195.80
97,220	621.50 (47.52)	686.16	1,307.66	136.67	202.15
103,320	621.50 (47.52)	686.16	1,307.66	136.67	202.15

[a] Figures in the parentheses indicate percentage of hired labor to total labor.

Labor in the Dike-Pond System of the Zhujiang Delta, South China

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RUDDLE, K. 1986. Labor in the dike-pond system of the Zhujiang delta, South China, p. 157-160. *In* J.L. Maclean, L.B. Dizon and L.V. Hosillos (eds.) The First Asian Fisheries Forum. Asian Fisheries Society, Manila, Philippines.

Abstract

There is little systematic information on the labor absorption capacity of integrated systems of agriculture and aquaculture. This hampers evaluation of their development potential, particularly in areas of severe population pressure. In the densely populated Zhujiang (Pearl River) Delta of South China an old-established dike-pond system based on the integrated production of Chinese carps, sugar cane, mulberry leaf, silkworms, and several minor commodities, has been acclaimed as an important absorber of rural manpower. This paper describes a simplified field methodology that was applied in a representative tract of that dike-pond system in Shunde County, to provide a rapid assessment of the labor demand and supply situation. It was found that 57% of the potential manpower available in the 20-39 years age group is absorbed by the system, and therefore that household economies are heavily dependent on income derived from jobs outside the system.

Introduction

Although integrated systems of agriculture and aquaculture are widespread in Asia they are not well known scientificallly. Their socioeconomic aspects are complicated and little understood. Moreover, since these systems often exist where population pressures are intense, their labor demands and ability to absorb manpower require detailed analysis. Such basic information is urgently needed for a representative range of integrated systems to plan their further development and to effect successful technology transfer.

This paper summarizes the labor demand of and supply to the large-scale dike-pond system of the Zhujiang Delta of Guangdong Province, South China (Ruddle 1985a), which has been characterized as having a high labor demand and therefore of being a critical generator of rural employment in an area of acute population pressure (e.g., Luo et al., unpublished data; Zhong, unpublished data). The research was conducted in the First Production Team, Nanshui Brigade, Leliu Commune, Shunde County, in 1982 and 1983.

The Dike-Pond System

The dike-pond system of the Zhujiang Delta is elaborately integrated and intensive, and is based on the production of mulberry *(Morus atropurpurea)*, silkworms *(Bombyx mori)*, sugar cane *(Saccharum officinarum)*, elephant grass *(Pennisetum purpureum)* (for fish feed), a range of vegetables, and the polyculture of Chinese carps (grass carp *[Ctenopharyngodon idella]*, silver carp *[Hypophthalmichthys molitrix]*, bighead carp *[Aristichthys nobilis]*, black or snail carp *[Mylopharyngodon piceus]*, mud carp *[Cirrhinus molitorella]* and common carp *[Cyprinus carpio]*), together with other minor crops. Average production rates (t/ha) are fish, 7.5; sugar cane, 75; mulberry leaves, 30; vegetables, 3.75; silkworm cocoons, 2.1 (per ha of mulberry); and elephant grass, 225. The dike-pond system is now the predominant agro-ecosystem over some 800 km^2 south of Guangzhou where it supports a population of about 1.2 million (Ruddle et al. 1983; Ruddle and Zhong, in press).

Methods

The labor times for each task required to produce a farm commodity, in man-hours/day/mu (1 mu = 0.066 ha), for the entire dike-pond system, which had been established empirically by the Nanshui Brigade for the allocation of work points, were used to measure labor demand. These were verified with specialists of the Leliu Commune Agricultural Research Station and sampled householders. Data were converted to man-days (md)/ha/year/task, then summed and multiplied by the area of each component of the system.

The labor supply calculations are based on 305 days/year of potential full-time labor (with 60 days allowed for holidays or festivals). Monthly labor demand is based on 25.4 working days/month. The annual full-time labor supply is calculated by multiplying the number of full-time workers by 305. Labor absorption capacity is expressed as the ratio between labor demand and the manpower potentially available to fulfill it.

Labor Demand of the System

A total of 905.2 md/ha/year is required to operate a composite hectare composed of the four basic components (fish, sugar cane, mulberry and silkworms) of the system (sum of component totals divided by 4), or the full-time annual input of three workers. One ha of mulberry requires 1,510 (5 workers), sugar cane needs 1,199 (4 workers), fishponds 536 (the equivalent of 1.7 workers), and silkworm-rearing (per ha/mulberry) 375 (the equivalent of 1.2 workers). Peak labor demand occurs between April and September (lunar reckoning) when 63.9% of the total input is made. October and November are relatively slack with only 4.6% and 3.9%, respectively.

Labor Input by the First Production Team

The demographic characteristics of the First Production Team (Table 1) are typical of the region.

Operation of the 9.97-ha dike-pond formerly assigned to this team required a total labor input of 18,133 md/year, or 60 full-time workers: mulberry required 4,289 (14 workers), sugar cane 7,435 (25), fishponds 5,344 (17) and silkworms 1,065 (3). The maximum labor input, 2.076 md, was made in April (82 workers), and the minimum, 534 md, in November (21 workers). The average monthly labor demand was 1,511 md (59 workers).

The system demanded the full-time input of far fewer workers than were potentially available to operate it, as the potential full-time labor supply of the 104 persons in the 20-39 years age group alone was 31,692 md/year. Thus the system could absorb only 57% of the potential full-time labor in this group. This is marked in the relatively slack season: in November, for example, when only 534 (20%) of the 2,641 md potentially available from this age group was absorbed. On the other hand, in April, August and December, the months of peak demand, 79%, 85% and 76%, respectively, of the potential labor of this 20-39 years age group was absorbed (Fig. 1 and Table 2).

Thus both full-time and seasonal employment outside the immediate system is of major importance (Table 1). This is exemplified below by one typical household.

Labor Requirements and Supply in a Typical Household

Under the Household Responsibility System of farming progressively implemented in the First Production Team since 1979 (Ruddle 1985b), individual households make contracts with the team to cultivate ponds and dikes. Depending on household size, various combinations of pond and dike area are contracted. The typical household (Table 3) operates all system components.

The capacity of this large household to supply labor to its dike-pond operation far exceeds the demand of 552 md/year. The aggregate potential input capacity of the six younger adult members is 1,830 md/year, of which the household's operation can absorb only 30%. Thus chronic underemployment has been overcome by having three family members fully employed outside the system (Table 3). Nevertheless, there remains an excess labor capacity in the household, which can potentially supply a further 1,278 md/year to the system or to other economic activities (householder, his wife and fifth son 305 md each, and the first and fourth sons and daughter-in-law 156.5 md each [60 free days plus 3 hours/day for 305 days]).

Discussion

The simple field methodology described provided a rapid and comprehensive assessment of the labor demand and absorption capacity of one complex integrated system, the dike-pond system of the Zhujiang Delta, Guangdong Province, South China, as operated in a representative tract of Shunde County. Although inherently labor intensive, research revealed that under prevailing demographic conditions the dike-pond system can absorb only 57% of the potential manpower available within the 20-39 years age group alone. (Since comparable data on other integrated systems are lacking, however, it is as yet impossible to rank the labor absorption capacity of this system.) It provides full-time employment for most of the able-bodied males and females, as those in the 20-39 age group undertake most of the heavy labor. Older people engage in more highly skilled but lighter tasks. Most households are heavily dependent on local jobs outside the system, which in some cases provide 50% of total net income (Ruddle 1985b).

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Table 1. Population characteristics and labor division in the first production team, Nanshui brigade.

Age group	Total	%	Male	Female	Work performed Male	Work performed Female
0-14	100	32.15	48	52	PS, S	PS, S
15-19	32	10.28	15	17	FP, S, SW	M, RW, S, SW
20-24	33	10.61	16	17	FP, SW	FP, RW
25-29	29	9.32	15	14	FP, SW	RW, SC
30-34	26	8.36	11	15	FP, SW	V
35-39	16	5.14	6	10	FP, SW	OW
40-44	12	3.85	3	9	SC	OW
45-49	10	3.21	5	5	SC	M, SC
50-54	16	5.14	7	9	OSL	M, SC
55-59	9	2.89	4	5	OSL	R
60+	28	9.00	13	15	R	R
Total	311	100.00	143	168	–	–

FP = fishpond; M = mulberry; OSL = outside side work; OW = outside work; PS = preschool; R = retired; RW = roadwork; S = school, SW = silkworm; SC = sugar cane; V = vegetables.

Table 2. Labor absorption capacity of the dike-pond system for the 20-39 years age group.

Month	Demand Man-days	Potential supply Total	Potential supply Male	Potential supply Female	Absorption rate Total	Absorption rate Male	Absorption rate Female
Jan	1,666	2,641	1,219	1,422	63	–27	–15
Feb	1,273	2,641	1,219	1,422	48	–04	–11
Mar	1,443	2,641	1,219	1,422	55	–15	100
Apr	2,076	2,641	1,219	1,422	79	–39	–31
May	1,818	2,641	1,219	1,422	69	–32	–22
Jun	1,546	2,641	1,219	1,422	59	–21	–08
Jul	1,474	2,641	1,219	1,422	56	–17	–04
Aug	2,232	2,641	1,219	1,422	85	–45	–39
Sep	1,329	2,641	1,219	1,422	50	–08	93
Oct	752	2,641	1,219	1,422	29	62	53
Nov	534	2,641	1,219	1,422	20	43	38
Dec	1,991	2,641	1,219	1,422	76	–38	–29

Table 3. Membership and occupations in a sample household.

Relationship to head	Age	Full-time occupation	Part-time occupation
Householder	63	Silkworms	Fishpond
Wife	60	Silkworms	Fishpond
First son	34	Factory labor	Fishpond
Daughter-in-law	32	Brickworks labor	General farm work
Fourth son	27	River transport	General farm work
Fifth son	19	General farm work	House building
Granddaughter	10	School pupil	Pig-raising
Grandson	8	School pupil	None
Granddaughter	5	School pupil	None

Table 4. Summary of farm labor demand in a sample household.

Component	Area (ha)	Labor demand (md/yr)	Full-time labor equivalent (%)
Pond	0.198	106	34
Sugar cane	0.165	198	64
Mulberry	0.132	199	65
Silkworms	–	375	49
Total	0.495	552	180

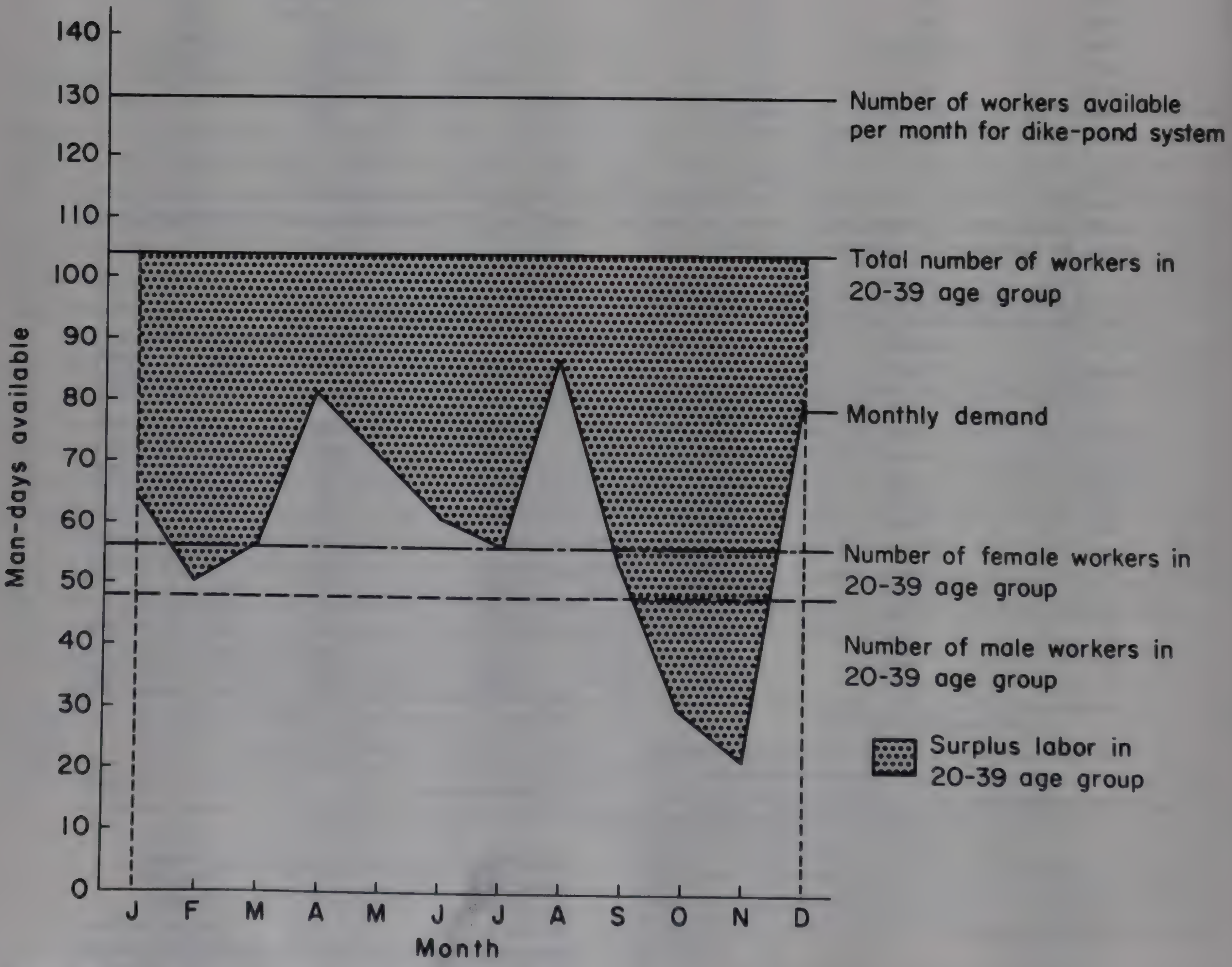

Fig. 1. Monthly labor availability and labor demand of the dike-pond system of the first production team, Nanshui Brigade.

Protein Moiety of the Biliverdin-Protein Complex in the Blood Serum of *Clinocottus analis* (Cottidae)

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Abstract

The chromoprotein responsible for the blue-green coloration in the serum of *Clinocottus analis* was precipitated from solution by salting out at 70% to 80% saturation with ammonium sulfate. In polyacrylamide gel electrophoretic analysis, the chromoprotein migrated ahead of most other proteins, just following the albumin band, and two green bands were observed. The isoelectric focusing point of the major green band was 3.6, while that of the minor green band was 4.6. SDS-polyacrylamide electrophoresis showed two major bands of molecular weight, 42,500 and 27,500, and one light band of molecular weight, 22,500. These results suggest that the protein moieties of the biliprotein in the blood serum of *C. analis* have characteristics similar to those of the blood albumin family.

Introduction

The biliverdin protein complex found in the blue-green blood serum of *Clinocottus analis* (Cottidae: Scorpaeniformes), the wooly sculpin, has been demonstrated to have an unusually strong bile pigment-protein binding capacity (Fang 1985a). Bile pigments that exist in the blood of mammals have been reported to be bound mainly to serum albumin (Blanckaert 1979). In these complexes, the binding character is quite different from that of the biliverdin-protein found in fish serum (Fang et al. 1986). But the identity of this binding protein has not been revealed before.

There have been several scattered studies on the characterization of the carrier protein in the fish serum biliprotein complex yet without general understanding. Bada (1970) studied the amino acid composition and estimated the molecular weight (MW) of the green serum chromoprotein of the arctic sculpin *(Myoxocephalus scorpioides)*. Low and Bada (1974) made MW determination on the blood chromoprotein of *Scorpaenichthys marmoratus*. Yamaguchi and Hashimoto (1986) studied the amino acid composition and MW of the biliverdin-protein from the blood serum of an eel *(Anguilla japonica)* and identified it as a β_1-lipoprotein. But this was reclassified as a serum albumin by Fang (1985b).

In this research, the general chemical and physical characteristics of the serum biliprotein of *C. analis* were studied. These results were further compared with those from other fishes.

Materials and Methods

C. analis weighing 15-26 g were collected from tide pools. They were kept in running seawater aquaria for 24 hr without feeding. Blood samples were drawn by cardiac puncture and the blue-green serum was obtained by immediately centrifuging the heparinized blood pooled from several fish.

For the fractionation test, crude blood serum ($\approx$1 ml) was diluted with 0.1 M phosphate buffer (pH 7.4) to a total volume of 10 ml. Solid ammonium sulfate was gradually added while the solution was stirred. At each step of saturation, half an hour stirring was allowed to achieve equilibrium before centrifugation. After centrifuging, the absorbance of the supernatant at 675 nm was measured by a Beckman Acta MVI spectrophotometer to monitor the presence of biliproteins.

Disc electrophoresis was carried out as follows. Chromoprotein obtained by back-extraction fractionation with ammonium sulfate (Cooper 1977) was redissolved in 1 ml of pH 7.4 phosphate buffer. The solution was then dialyzed overnight against the same buffer at 4°C, concentrated with a low pressure vacuum rotatory evaporator at room temperature and applied to disc polyacrylamide gel electrophoresis (12% gel, pH 8.3). Bovine albumin (Sigma Chemical Co.) and bovine albumin incubated with biliverdin (Sigma Chemical Co.) (1:1, molar ratio) were also analyzed to provide a basis for comparison of electrophoretic mobilities.

Following purification of the fish serum biliprotein by polyacrylamide gel electrophoresis, the green bands were cut off, eluted and then analyzed using SDS-polyacrylamide gel electrophoresis to estimate the molecular weight of the chromoproteins.

The electric current in electrophoresis was 3 mA/tube for disc gels and 5 mA/tube for SDS gels. After each run, the R_f of the green band was recorded; the band was marked with a pin and the gel stained for protein with Amido Schwarz.

The isoelectric point (pI) of the biliprotein was measured on a LKB-110 ml isoelectric focusing column. The sample was prepared for isoelectric focusing in the same way as those for electrophoresis. After a 24-hour analysis at room temperature, two sharp green bands appeared near the anode. Fractions were collected from the isoelectric focusing column and the pIs of the green components were estimated by measuring the pH and the absorbance at 675 nm of each fraction.

Amino acid compositional analysis was made as follows. Fish blood biliprotein, purified by electrophoresis, was dialyzed against distilled water overnight; the sample was dried by a vacuum rotator and then hydrolyzed for 24 hours in 6M HC1 at 100°C. After hydrolysis, the acid was removed by rotatory evaporation. The sample was then analyzed by a Beckman model 118 amino acid analyzer interfaced with a DEC PDP-11 computer for data acquisition and analysis.

Results

The green chromoprotein was precipitated at 70% to 80% ammonium sulfate saturation (Fig. 1). When this partly purified green chromoprotein was analyzed by disc gel electrophoresis, a green band was observed to closely follow the fastest migration albumin band. The green band migrated faster than the standard bovine albumins (Fig. 2). The R_f for bovine albumin incubated with biliverdin was 0.32; for bovine albumin alone, 0.31 and for the green band, 0.37.

After running the native gel longer to achieve better resolution, two green bands were separated (Fig. 2d). The major band migrated faster, with the minor one following closely behind. Thus, there appears to be two forms of the green chromoprotein, or possibly the chromoprotein present in the fish serum is actually a dimer.

In the isoelectric focusing experiment, two green bands were observed near the anode after the 24-hour analysis. One major green band appeared at ~ pH 3.6, and a minor green band appeared around pH 4.6 (Fig. 3).

Three bands appeared in SDS-polyacrylamide gel electrophoresis. Two major bands showed a protein molecular weight (MW) of 42,500 and 27,500, respectively. One light band with a MW of 22,500 was also present.

The amino acid composition of the purified biliprotein is given in Table 1 as are the data for bovine albumin and the biliprotein present in the serum of the eel, *Anguilla japonica* (Fang 1985b). The presence of large amounts of aspartic acid and glutamic acid in the biliprotein from *C. analis* is consistent with the low pI determined for the biliprotein. There are considerable differences in amino acid composition between bovine serum albumin and that of the biliprotein of *C. analis* despite · the general similarity of physicochemical characteristics of the two proteins.

Discussion

Most blood plasma globulins are salted out at less than 50% ammonium sulfate saturation, whereas albumins and some euglobins precipitate at higher saturations (Canatarow and Schepartz 1967). The electrophoresis data obtained in this research suggest that the biliverdin carrier protein in the blood of *C. analis* is not a euglobin but resembles albumin since the green bands and albumin had similar electrophoretic mobilities (Figs. 2a and 2b). If the biliprotein in *C. analis* serum were euglobin, it would have moved much more slowly than albumin (Longsworth 1942; Orten and Neuhaus 1970).

The electrophoresis results indicate that there are at least two types of green chromoproteins in the blood of *C. analis*. Both proteins differ from bovine serum albumin by having either a different electrophoretic mobility or a different pI (pI of albumin is 4.6 to 4.9), and by having a different MW (MW of albumin is 69,000). However, their precipitation at high ammonium sulfate saturation, fast electrophoretic mobility and low pI are all characteristics of the albumin family. The carrier proteins for biliverdin in the blood of *C. analis* are thus possibly members of the serum albumin group.

The appearance of two green bands in acrylamide gel electrophoresis, two to three peaks in pI analysis, and two major and one minor band in the SDS-gel electrophoresis all indicate the existence of at least two different proteins in the green chromoprotein of *C. analis* blood serum. One of the green bands of the fish serum has a pI around 4.6, the same as that of albumin. Therefore it could be that one of the green bands is merely a biliverdin-albumin complex with an electrophoretic mobility slightly different from that of albumin. However, this is apparently not the case because the R_f of the biliverdin-albumin standard had an R_f different from that of the green biliprotein; the electrophoretic mobility of the carrier protein is also the same with or without biliverdin attached (Fang 1985a). If the carrier protein was merely an albumin, the liberated protein moiety should have a mobility on polyacrylamide gels similar to other fish serum albumins (Komatsu et al. 1970).

Low and Bada (1974) suggested that the blue-green coloration of the serum of *C. analis* is due to the presence of only one pigment protein and is generated by biliverdin in two distinct micro-environments on the same protein.

The results obtained here do not rule out this possibility, although it appears more likely that two or more distinguishable protein moieties bind biliverdin in *C. analis* blood.

The fractionation data of the chromoprotein isolated from *C. analis* agree very well with those of the chromoprotein from the serum of the arctic sculpin *(M. scorpioides)*, and are similar to those of the eel *(A. japonica)*, but are different from the blue and green chromoproteins from the cabezon *(Scorpaenichthys marmoratus)* (20-40% and 50-65%, respectively) (Low and Bada 1974). The pI of the major chromoprotein of *C. analis* is 3.6. This is similar to the pI (3.3-3.7) of the green chromoproteins isolated from red blood cells of the skipjack tuna *(Katsuwonus pelamis)* (Fuke et al. 1973), but is higher than that from the arctic sculpin whose pI is 3.1. The pI of the minor chromoprotein of *C. analis* (4.6) is the same as that of the eel chromoprotein.

The MWs of the various green chromoproteins in the blood of *C. analis* were estimated to be 42,500, 27,500 and 22,500, respectively, in this research (Fig. 4). The MW of arctic sculpin serum chromoprotein was estimated to be 46,000 (Bada 1970) and that of the cabezon chromoprotein 47,500 (Low and Bada 1974). Kochiyama et al. (1966) found the MW of eel serum chromoprotein to be 89,100. The MWs of the three green chromoproteins isolated from red blood cells of skipjack were 4,000-6,000, 8,000-10,000 and 9,000-10,000 (Fuke et al. 1973). These results suggest that at least one of the green chromoproteins in *C. analis* serum (i.e., the one with pI -3.6, MW-40,000 to 50,000) may be present in the serum of other fish with blue-green blood serum.

The green chromoproteins from the blood of different fishes seem to have a general albumin-like character. However, their amino acid composition is different from bovine serum albumin. As there are few data about the amino acid composition of fish serum albumin, it is not clear whether the biliverdin carrier proteins are a somewhat modified fish albumin or whether the carrier proteins are a group of proteins unique to fish serum with the properties of strongly binding biliverdin (Fang 1985a; Fang et al. 1986).

The blue-green chromoproteins in the blood serum of *C. analis*, precipitated at 70%-80% saturation of ammonium sulfate, have pI values of 3.6 and 4.6, migrate very close to albumin in electrophoresis and have estimated MWs of 42,500, 27,500 and 22,500. These results suggest that the protein moiety of the blue-green chromoproteins in the blood of fish are not β_1-lipoproteins as previously thought, but are more similar to blood albumin type proteins.

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Table 1. Relative amino acid compositions of the serum biliprotein of *Clinocottus analis, Anguilla japonica* (data converted from Yamaguchi et al. 1968), and bovine serum albumin (data converted from Fasman 1977). All value are normalized to leucine. The value for glycine is not included because Tris-glycine buffer was used in the electrophoresis purification procedure and glycine contamination was likely.

Source Amino acid	Biliprotein isolated from *Clinocottus analis*	Bovine serum albumin	Biliprotein isolated from *Anguilla japonica*
Asp	2.021	0.868	1.306
Thr	0.998	0.524	0.613
Ser	1.110	0.426	0.687
Glu	1.539	1.129	1.318
Ala	0.792	0.754	0.723
Val	0.558	0.573	0.709
Ileu	0.610	0.213	0.473
Leu	1.000	1.000	1.000
Tyr	0.505	0.312	0.442
Phe	0.907	0.426	0.451

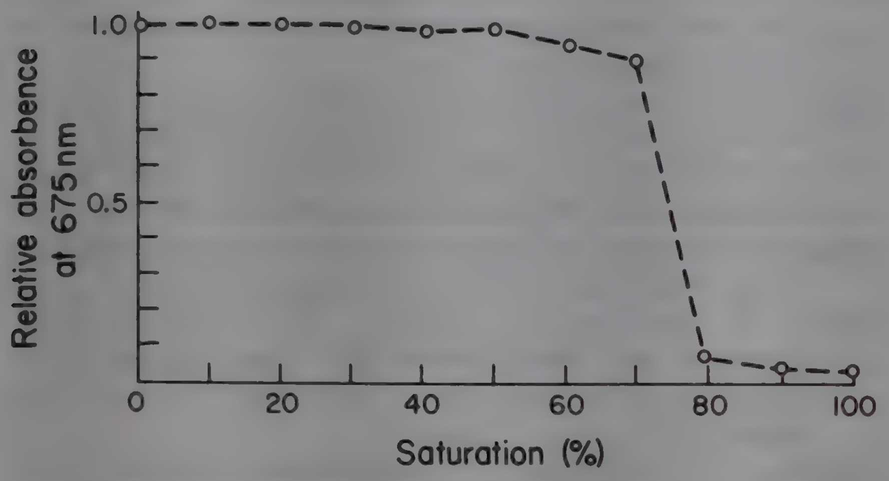

Fig. 1. Saturation-fractionation of the blue-green chromoprotein from the fish blood serum by ammonium sulfate.

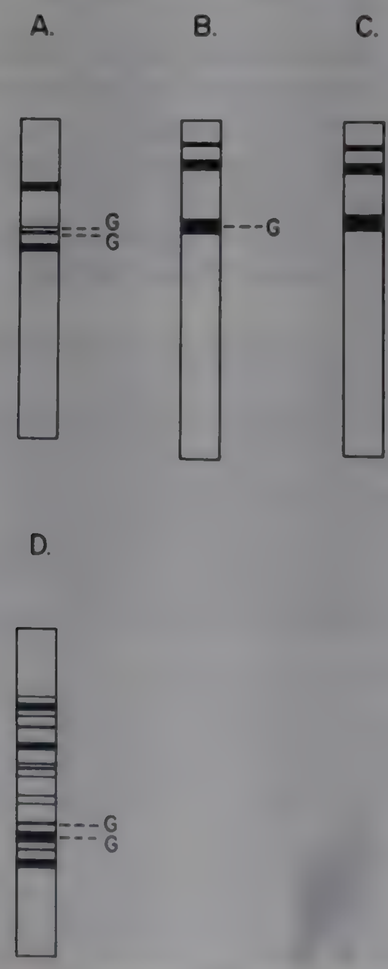

Fig. 2. Electrophoretic patterns of the blue-green blood serum chromoproteins of *C. analis*. A. Back-extraction fractionated blue-green fish serum. B. Bovine albumin incubated with biliverdin. C. Bovine albumin. D. High resolution of fractionated fish serum. The patterns were obtained by the projection of the gels. "G" represents locations of green bands.

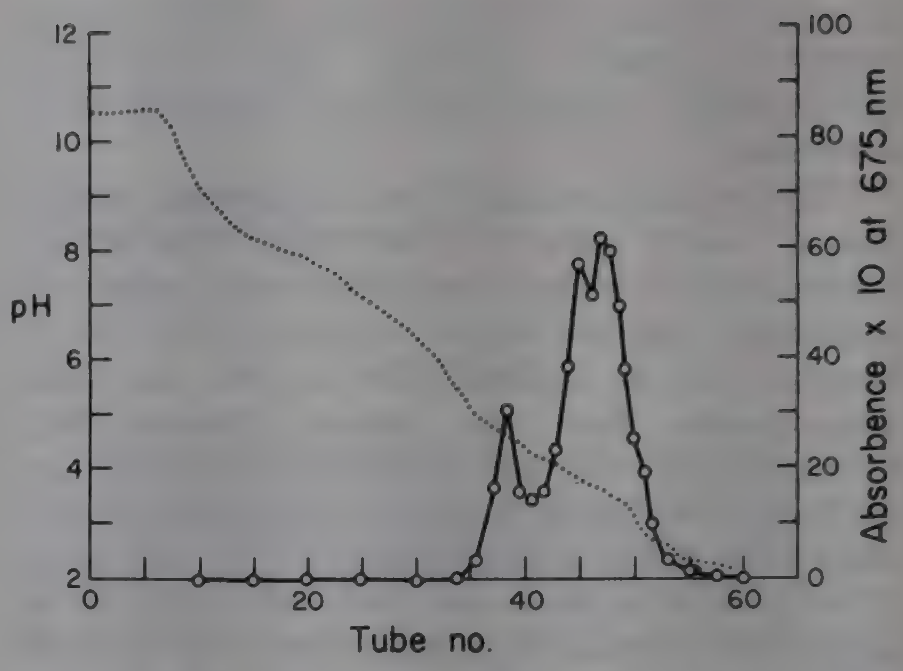

Fig. 3. The isoelectric focusing points of the blue-green fish serum chromoprotein. (●●●●●●), pH. (— ○ —), absorbence at 675 nm.

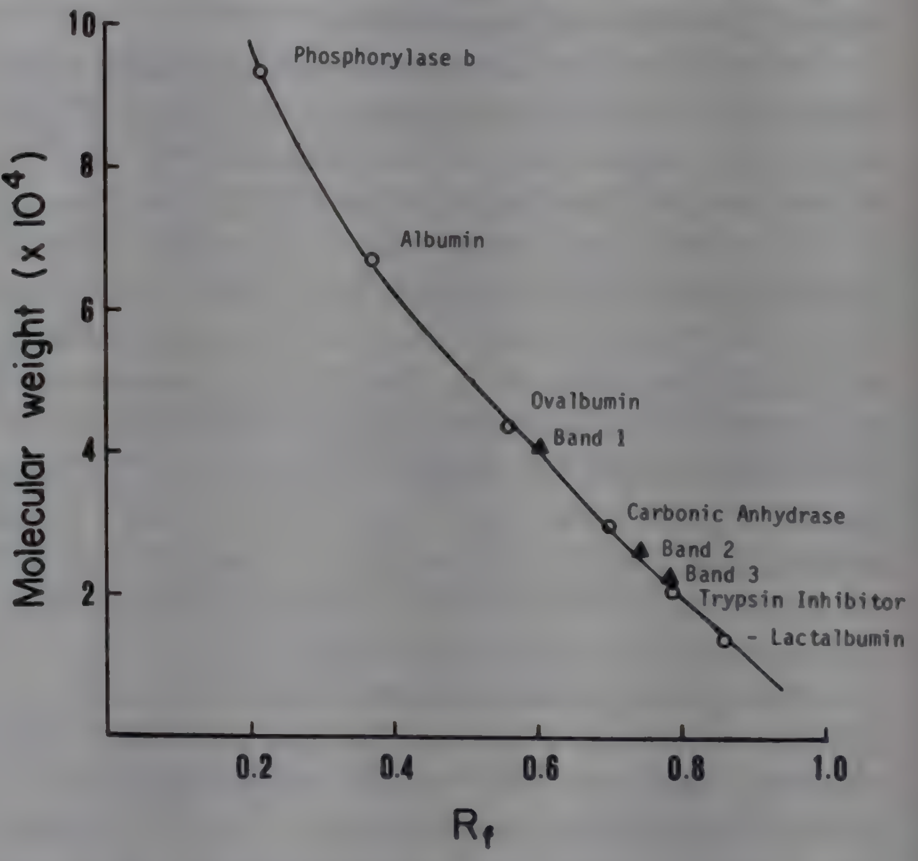

Fig. 4. The molecule weight of the three protein units from the blue-green serum chromoproteins of *C. analis*.

Polysaccharides of *Gelidium* spp.

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Abstract

Chemical studies on the polysaccharides from different stages of the life history of two *Gelidium* species of central Chile (Rhodophyceae) are presented. Fractionation of the polysaccharide (yield 16.6%, 3, 6-anhydrogalactose content 31.3%, sulphate 3.18%) from cystocarpic plants of *Gelidium lingulatum* afforded five fractions. Fraction 1 (28.3%, eluted with water), fraction 2 (34.9%, eluted with 0.1M KC1), fraction 3 (10.5%, with 0.4 M KC1), fraction 4 (18.5%, with 0.8 M KC1) and fraction 5 (7.8%, with 1.5 M KC1).

Polysaccharide (yield 26.7%, 3,6-anhydrogalactose content 43.0%, sulphate 3.00%) from tetrasporangial plants of *G. lingulatum* was fractionated affording fraction 1' (55.0%, eluted with water), fraction 2' (21.0%, eluted with 0.1 M KC1), fraction 3' (6.2%, with 0.4 M KC1), fraction 4' (13.2%, with 0.8 M KC1) and fraction 5' (4.5%, with 1.5 M KC1).

Seasonal variation in polysaccharide yield and gel strength were studied for *Gelidium* sp. For cystocarpic and vegetative plants, the highest yield in polysaccharides was in summer (37.6 and 40.0%, respectively) whereas for tetrasporangial plants the highest yield (23.8%) was in autumn. No relation was found between polysaccharide yield and gel strength. The highest values in gel strength were in agarans from samples collected in winter. Alkaline treatment of the polysaccharides increased (up to 1,000 g/cm^2) gel strength values in all the cases.

Introduction

In the rocky intertidal of central Chile, *Gelidium lingulatum* J. Agardh, *G. filicinum* Bory and *G. rex* Santelices occur at the same tidal level. These species are exported as raw material for agar production.

Agar is a complex mixture of polysaccharides extracted from red seaweeds (Rhodophyceae), mainly from the genera *Gracilaria, Gelidiella, Acanthopeltis, Pterocladia* and *Gelidium*. According to Duckworth and Yaphe (1971), agar is essentially a mixture of agarose, pyruvated agarose and sulfated galactans. Araki (1937) deduced the structure of agarose to be (1-4)-linked, 3,6-anhydro-α-L-galactose alternating with (1-3)-linked β-galactopyranose.

The amount of 3,6-anhydrogalactose and sulfate have an important influence on the gelling behavior of agar and hence on its economical value.

The phycocolloid from *Gelidium lingulatum* has been previously studied in this laboratory (Zanlungo 1980). It was shown that it is an agar-type polysaccharide.

In this paper the chemical studies on the polysaccharides from tetrasporangial and cystocarpic plants of *G. lingulatum* and of cystocarpic, tetrasporangial and vegetative plants of *G. rex* are presented.

Materials and Methods

The seaweeds were collected in Pupuya (34°S, 71°65'W) and were carefully sorted in the Laboratorio de Zoologia, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile. The general methods have been previously described (Klein et al. 1984). Gel strength of 1.5 % agar solution was measured using a Marine Colloids Gel Tester.

To extract the polysaccharides, dried ground seaweed (100 g) was stirred with water (3 l) at 95°C for 1 hour. The mixture was filtered through muslin and the extraction process was repeated twice. The resulting gel was frozen at -5°C and then thawed; this process was repeated twice affording a gel which was poured into acetone.

Sugar composition of samples was determined by total hydrolysis in 2M trifluoroacetic acid at 100°C for 16 hours. The liberated sugars were converted into alditol acetates and analyzed by gas-liquid chromatography. Fractionation of the whole polysaccharide was effected by using DEAE-Sephadex A50. The anion-exchanger was equilibrated in 1M HC1, washed with water and then packed into a column. A solution of the polysaccharide (250 mg) in warm water was applied to the column which was equilibrated at 65°C. The column was eluted with water and fractions (10 ml) were collected until the eluant became polysaccharide-free. A gradient concentration of potassium chloride (0.1-1.5M) was then used to eluate more highly charged polysaccharides. The carbohydrate contents of the fractions were determined with phenol-sulfuric acid reagent.

Results and Discussion

Table 1 lists yield and analytical data found for agarans from *G. lingulatum.* It can be seen that the yields are quite different. According to the literature, the yield in agar from different agarophytes varies between 6 and 28.5% (Young et al. 1971). For unsorted *G. lingulatum* a yield of 18.1% has been reported (Zanlungo 1980).

Total hydrolysis of the polysaccharides followed by paper chromatography showed that galactose is the main component. Minor sugars in the hydrolyzates were identified by gas-liquid chromatography as alditol acetates. Results are shown in Table 2.

The 6-0-methylgalactose is a common sugar in agar-type polysaccharides. According to Araki et al. (1967) the amount of this sugar in agar from Japanese agarophytes varies between 0.8 and 21%. For unsorted *G. lingulatum,* Zanlungo found 7.6% 6-0-methylgalactose, 1.5% glucose, 0.9% 2-0-methylgalactose and 0.5% mannose.

The polysaccharides were fractionated by column chromatography using DEAE Sephadex (C1^{-}). Results are shown in Table 3.

It was found in each case, with increasing ionic strength of the eluant, that the sulfate content of the eluted polysaccharide increased. This result agrees with those reported previously in the literature for agar from some agarophytes (Young et al. 1971). No data are available for agar from karyologically different generations.

Homogeniety of the fractions was studied by polyacrylamide gel electrophoresis. It was found that all the fractions were heterogenous.

Fractionation of agarans from *Gelidium rex* gave similar results. For example, fractionation of the polysaccharide (yield 24.9%, sulfate 3.2%) from cystocarpic plants collected in autumn, afforded four fractions: fraction 1 (22.9%, eluted with water); fraction 2 (29.1%, eluted with 0.1M KC1); fraction 3 (18.0%, with 0.4M KC1); and fraction 4 (29.8%, with 0.8M KC1).

Seasonal variation in polysaccharide yield and gel strength were studied for *Gelidium rex.* For cystocarpic and vegetative plants, the highest yields of polysaccharides were found in summer (37.6 and 44%, respectively) whereas for tetrasporangial plants the highest yield (23.8%) was found in autumn. No relation was found between polysaccharide yield and gel strength. The highest values in gel strength were found in agarans from samples collected in winter as shown in Table 4.

If sulfate groups are located on carbon-6 in galactose residues in the polysaccharide, they can be converted into 3,6-anhydrogalactose by treatment with alkali, and an increase in gel strength results. Of the polysaccharides examined, that from vegetative plants of *G. rex* collected in autumn formed the strongest gel after treatment with alkali. The gel strength value was raised to 1,007.4 g/cm^{2}. After treatment with alkali, there was an increase in the content of 3,6-anhydrogalactose and a decrease in sulfate content (Table 5).

The results so far obtained in this work indicate that the phycocolloids from different nuclear phases of *G. lingulatum* and *G. rex* are complex mixtures of polysaccharides composed of a series of related polymers which range from a "neutral" fraction to highly charged galactans. Therefore, no relation between the chemical structure of the agar and the stage of the life history of the algae can be proposed.

Acknowledgements

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Table 1. Yield, gel strength and composition for polysaccharides from *Gelidium lingulatum.*

	Yield %	Gel strength g/cm^2	3,6-anhydro-galactose %	Sulfate as SO_3 %
PST[a]	26.7	694	43.0	3.00
PSC	16.6	365	31.3	3.18

[a] PST = Polysaccharide from tetrasporangial plants.
PSC = Polysaccharide from cystocarpic plants.

Table 2. Total hydrolysis of polysaccharides from *G. lingulatum.*

Monosaccharide constituents.

Alditol acetate	PST[a] %	PSC[b] %
Galactitol	91.2	91.4
6-0-Methylgalactitol	2.5	3.4
2-0-Methylgalactitol	2.9	–
Glucitol	2.5	–
Xylitol	0.7	1.1
Unidentified	–	3.5

[a] PST = Polysaccharide from tetrasporangial plants.
[b] PSC = Polysaccharide from cystocarpic plants.

Table 3. Composition and yield of the polysaccharide fractions from *G lingulatum.*

Fraction	Eluant	Yield %	Galactose %	3,6-anhydro-galactose %	Sulfate as SO_3 %
From tetrasporangial plants					
I	Distilled water	55.0	45.24	42.14	2.0
II	0.1M KC1	21.0	48.94	38.00	3.6
III	0.4M KC1	6.2	43.94	25.53	5.2
IV	0.8M KC1	13.2	–	27.89	7.1
V	1.5M KC1	4.5	57.41	20.82	9.9
From cystocarpic plants					
I	Distilled water	28.3	53.87	39.15	3.4
II	0.1M KC1	34.9	47.00	38.15	4.6
III	0.4M KC1	10.5	51.20	31.77	6.2
IV	0.8M KC1	18.5	51.16	38.89	8.9
V	1.5M KC1	7.8	43.15	37.42	12.8

Table 4. Yield and gel strength for polysaccharides from *G. rex* collected in winter.

	Vegetative	Cystocarpic	Tetrasporangial
Yield (%)	26.7	34.3	14.2
Gel strength (g/cm^2)	595	553	700

Table 5. Analytical data for polysaccharides before and after treatment with NaOH.

Polysaccharide from tetrasporangial	Gel strength g/cm^2	3,6-anhydro-galactose %	Sulfate as SO_3 %
G. rex	553.7	36.9	2.9
Alkali treatment *G. rex*	749.7	45.4	2.4
G. lingulatum	694.0	43.0	3.00
Alkali treatment *G. lingulatum*	740.0	44.0	2.8

Gill Development in the Cichlid *Oreochromis niloticus*

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Abstract

Development of gills in *Oreochromis niloticus* (L.) from the fry to adult stage was studied. Relationship between total gill area (A) and body weight was a double logarithmic curve of the form $A = aW^b$. The slope 'b' was 0.870 and the intercept 'a' 3.519. This is similar to the gill dimensions of other fishes. Since the slope for gill area/weight curve is similar to that of oxygen consumption/weight curve it is postulated that *O. niloticus* is a fairly active fish species. Gills increase in area by increasing length of filaments and area of secondary lamellae. As the fish increases in size, the number of secondary lamellae mm^{-1} of filaments also decreases as noted in other fish species. The volume of water flowing over the gills was also calculated using the Poiseuille equation. The possible significance of gill development in relation to metabolism is also discussed.

Introduction

Research on fish gills has become increasingly important in recent years in view of their role in gas exchange and consequently their susceptibility to environmental pollutants (Karlsson 1983). Thus, an estimate of gill lamellar area is important because gills are the actual sites of gas exchange. Such studies, referred to as gill morphometry, have been confined mainly to temperate species (Hughes 1984), while studies on tropical species are very few. In addition, studies on gill development have been made only on four temperate species: smallmouth bass, *Micropterus dolomieu* (Price 1931); herring, *Clupea harengus* and plaice, *Pleuronectes platessa* (De Silva 1974) and carp, *Cyprinus carpio* (Oikawa and Itazawa 1985). This study was initiated as part of a wider program on the biology of *Oreochromis niloticus* (L.) and its potential in aquaculture.

Materials and Methods

Gill measurements were made on *O. niloticus* specimens of 4 mg-330 g fixed in 10% formalin for three weeks. Total length and weight were determined after fixation in formalin and prior to determinaton of gill areas. The method of Hughes (1966) where detailed measurements on the second arch was made. The lengths of every 5th filament and secondary lamellar area were determined at three points specified by Hughes (1966).

Since the gills at fry stages were still developing, the following method was used. Total length of every 5th filament was measured and spacing of lamellae and secondary lamellar area determined at three points on the filament. The area of a secondary lamella at each point was determined as the area of a triangle where the height and length of lamella was measured. The values for the gill area of each arch were then summed up to give the total gill area for that specimen.

Results

The changing length of gill filaments on all four arches of *O. niloticus* is shown in Fig. 1. It is evident that in arch I the filaments of the posterior hemibranch are longer on the first two-thirds of the arch than on the anterior hemibranch, whereas in arch II they are almost equal. In arch III the anterior filaments are longer than the first two-thirds of the gill arch. In arch IV the filaments of the anterior hemibranch are very long, in keeping with the movement of water through the pharynx.

The gill area to weight relationship can be denoted as a double logarithmic one (Hughes 1966). Fig. 2 shows the relationship between gill area and weight. These data are grouped into length classes (Table 1). While gill area increases with size of fish, considered as a ratio to its weight it decreases with age.

Fig. 3 shows the change in various parameters of gill dimensions with increasing size. Total filament length (Fig. 3a) increases at a more rapid rate than the total number of filaments (Fig. 3b). Fig. 3d shows that initially the number of secondary lamellae/mm of filament increases rapidly until the fish is about 1 g in weight and then decreases with increasing weight, showing allometric growth with development, although a single line is drawn for convenience since such a change is not evident when the gill area/weight relationship is considered. Similarly, individual secondary lamellar area also appears to decrease gradually with a change in the slope around 1 g weight though a single line is drawn for convenience. However, gill area increases mainly by increasing filament length (Fig. 3a) and, therefore, by increasing the number of secondary lamellae.

Table 2 gives the values for the intercept (a) and regression coefficient (b) for the total gill area and its component parameters, plotted on log/log coordinates as in Fig. 2. The general equation for such relationships is $Y = aW^b$ or $\log Y = \log a + b \log W$.

Data were grouped into length classes (Table 3) and dimensions of the gill sieve determined by using the Poiseulle equation (Hughes 1966). It is evident that 1/d', the number of secondary lamellae/mm of filament, decreases very rapidly from about 60 to 17 with increasing size of fish over the size range studied. However, as mentioned earlier, in Fig. 3d, this is not the case in the early stages.

Discussion

The decrease in gill area-unit weight with development has also been observed by several workers (Price 1931; Hughes 1966; Oikawa and Itazawa 1985) and is believed to be due to a decrease in actively respiring tissue with age (Zeuthen 1953). The slope of the gill area/weight relationship is also similar to that observed in small mouth bass (Price 1931) where a slope of 0.78 is observed, while in the present study it was 0.87. Muir and Hughes (1969), working on tunas, obtained values of 0.85 for the slope for the gill area/weight relationship in all three species of tunny, but for the bluefin alone it exceeded 0.9. Oikawa and Itazawa (1985) observed a triphasic allometry between gill area and weight in carp ranging from 7.07 in early stages to 0.79 in the adult stage while De Silva (1974) observed a change in the slope in the gill area/weight relationship from 3.36 in the larval stage to 0.78 after metamorphosis in herring and from 1.59 to 0.85 before and after metamorphosis in plaice. Such changes in the slope were not observed in the present study for the gill area/weight relationship but could be seen in the relationship between total filament length and weight and also in the relationship between the area of an average secondary lamella with development (Figs. 3c and d).

A 100-g *O. niloticus* has a gill area of 7.7×10^4 mm^2 which is about 3.5 times the value of a toadfish of similar weight (Hughes and Gray 1972) and half that of a very active fish such as *Trachurus trachurus* of 125 g (Hughes 1966). The gill area per unit weight in *O. niloticus* ranges from 550 to 730 for fish above 100 g. In toadfish (Hughes and Gray 1972) it ranges from 217 to 205 for fish of 100-300 g while in highly-active fishes such as menhaden and mackerel it is over 1,000 (Hughes 1966). These findings show that *O. niloticus*, although considerably more active than the toadfish, is nevertheless less active than species like the menhaden and mackerel. Decrease in spacing of lamellae with increasing size has been observed for many species (Price 1931; Hughes 1966; Muir and Hughes 1969). Price obtained a decrease from 28 to 17.8 for fish of 0.33-837 g in *Micropterus dolomieu* while Oikawa and Itazawa (1985) also showed a decrease in spacing in adult carp but an increase in larval stages.

Since the gill area/weight relationship is believed to be involved with active metabolism rather than resting metabolism (Oikawa and Itazawa 1985) the high slope value in this study could be indicative of high activity in this species. This is borne out by the studies of De Silva et al. (1986) which obtained values of 0.986 for the slope of the oxygen consumption/weight relationship of routine active fry of *O. niloticus*. This is comparable to the gill area/weight relationship of this species in this study.

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Table 1. Average length, weight and gill area for each 25-mm length class of *O. niloticus.*

Length class (mm)	Length (mm)	Weight (g)	No.	Gill area (mm²/fish)	(mm²/g)
1 – 24	13.11	0.049	(2)	114.59	2,314.96
25 – 49	29.00	0.414	(4)	752.76	1,818.25
50 – 74	54.00	2.220	(1)	1,090.61	761.40
75 – 99	84.60	12.229	(3)	5,416.21	442.82
100 – 124	111.66	25.233	(3)	17,776.72	704.50
125 – 149	136.25	47.740	(4)	40,358.50	845.83
150 – 174	166.00	85.330	(3)	63,784.60	747.48
175 – 199	192.66	140.660	(3)	78,961.42	561.34
200 – 224	212.50	196.000	(2)	129,293.96	666.36
225 – 249	229.60	268.000	(5)	162,638.30	606.86
250 – 274	258.50	258.000	(7)	190,314.70	737.65
275 – 299	–	–	–	–	–
300 – 324	306.00	492.000	(1)	354,416.45	720.36
325 – 349	330.00	712.000	(1)	411,466.00	577.90

Table 2. Regression analysis of dimensions of gills.

	Intercept (a)	Slope (b)	Correlation coefficient (r)	95 C.L. of b. Probability 1
Gill area (mm²)	3.519	0.8703	0.990	0.860 < b < 0.902
Total number of filaments/fish	179.300	0.2282	0.992	0.220 < b < 0.236
Total filament length (mm)	20.39	0.564	0.997	0.554 < b < 0.575
Area of secondary lamellae (mm²)	1.145×10^3	0.3982	0.979	0.377 < b < 0.420
Height of secondary lamellae (mm)	0.026	0.204	0.978	0.106 < b < 0.303
Length of secondary lamellae (mm)	0.0588	0.167	0.956	0.094 < b < 0.279
Number of secondary lamellae/mm	89.53	–0.119	–0.780	–0.191 < b < –0.047

Based on $Y = aW^b$ The correlation coefficient (r) has been calculated for the same data based on 59 specimens of *O. niloticus.*

Table 3. Dimensions of the gill sieve of different sizes of *O. niloticus.*

Length class (mm)	Mean weight (g)	Filament length (mm)	a	d^1 (cm)	Height of lamella (b) (cm)	Length of lamella (1) (cm)	q (cc/pore/cm.H_2O/sec) ($\times 10^4$)	Water flow through gills (Nq)—cc/sec /cm.H_2O
1 – 24	0.048	172.5	58.2	0.00196	0.0040	0.0089	0.3525	0.3538
25 – 49	0.414	684.8	60.7	0.00280	0.0084	0.0122	1.0399	4.32
50 – 74	2.220	2,606.4	28.2	0.00308	0.0067	0.0138	1.4776	10.858
75 – 99	12.226	4,374.2	21.2	0.00308	0.0100	0.0206	1.4774	13.70
100 – 124	25.233	6,517.0	25.0	0.00308	0.0138	0.0289	1.4533	23.67
125 – 148	47.740	11,261.0	23.4	0.00336	0.1720	0.0342	1.9872	52.36
150 – 174	85.333	11,745.4	22.9	0.00336	0.0227	0.0410	2.1877	58.83
175 – 179	140.666	14,078.0	20.9	0.00336	0.0232	0.0459	1.9972	58.76
200 – 224	196.000	18,140.0	20.2	0.00364	0.0263	0.0533	2.4789	90.84
225 – 249	268.000	22,787.4	21.1	0.00364	0.0244	0.0527	2.3260	111.84
250 – 274	258.000	21,594.6	19.6	0.00364	0.0294	0.0598	2.4699	104.53
275 – 299	–	–	–	–	–	–	–	–
300 – 324	492.000	30,512.4	16.8	0.00364	0.0368	0.0752	2.4584	126.02
325 – 349	712.000	32,512.4	16.6	0.00392	0.0386	0.0788	3.0736	165.88

The rate of water flow (q) is calculated from the Poiseuille Equation of Hughes (1966) where

$$q = \frac{P_1 - P_2}{n} \quad \frac{5d^3b}{24\ 1}$$

a—number of lamellae per mm of filament; d^1—distance from the middle of one secondary lamella to the middle of the next.

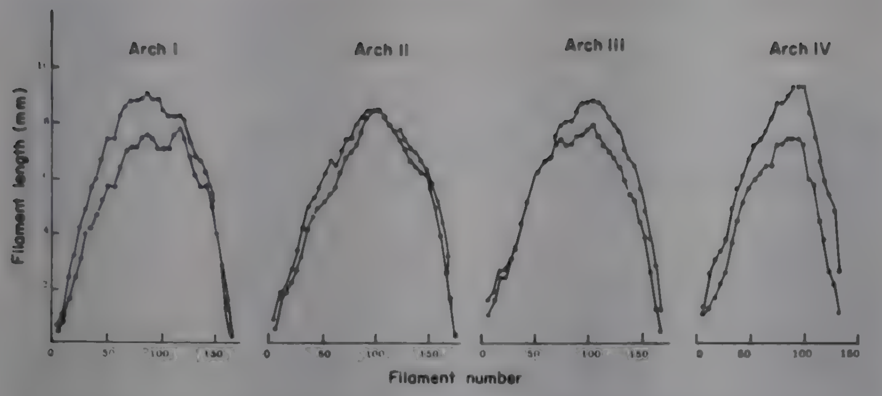

Fig. 1. Filament length on different gill arches of the right side of *O. niloticus*. Solid symbols indicate length of filaments of the posterior hemibranch in each arch.

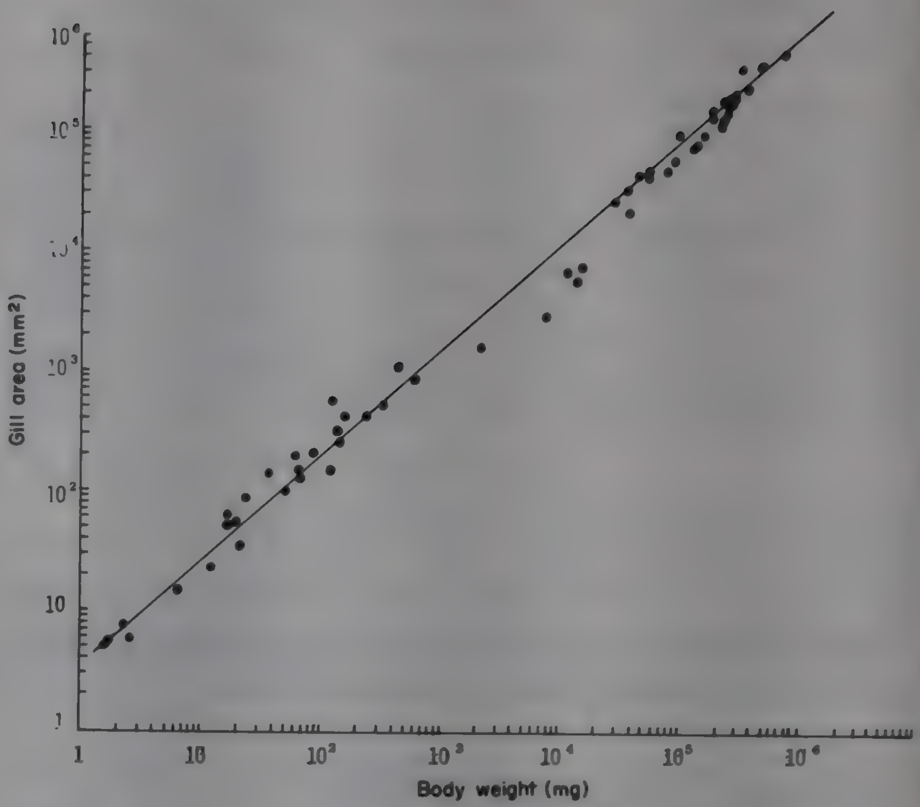

Fig. 2. Relationship between gill area and weight in *O. niloticus*.

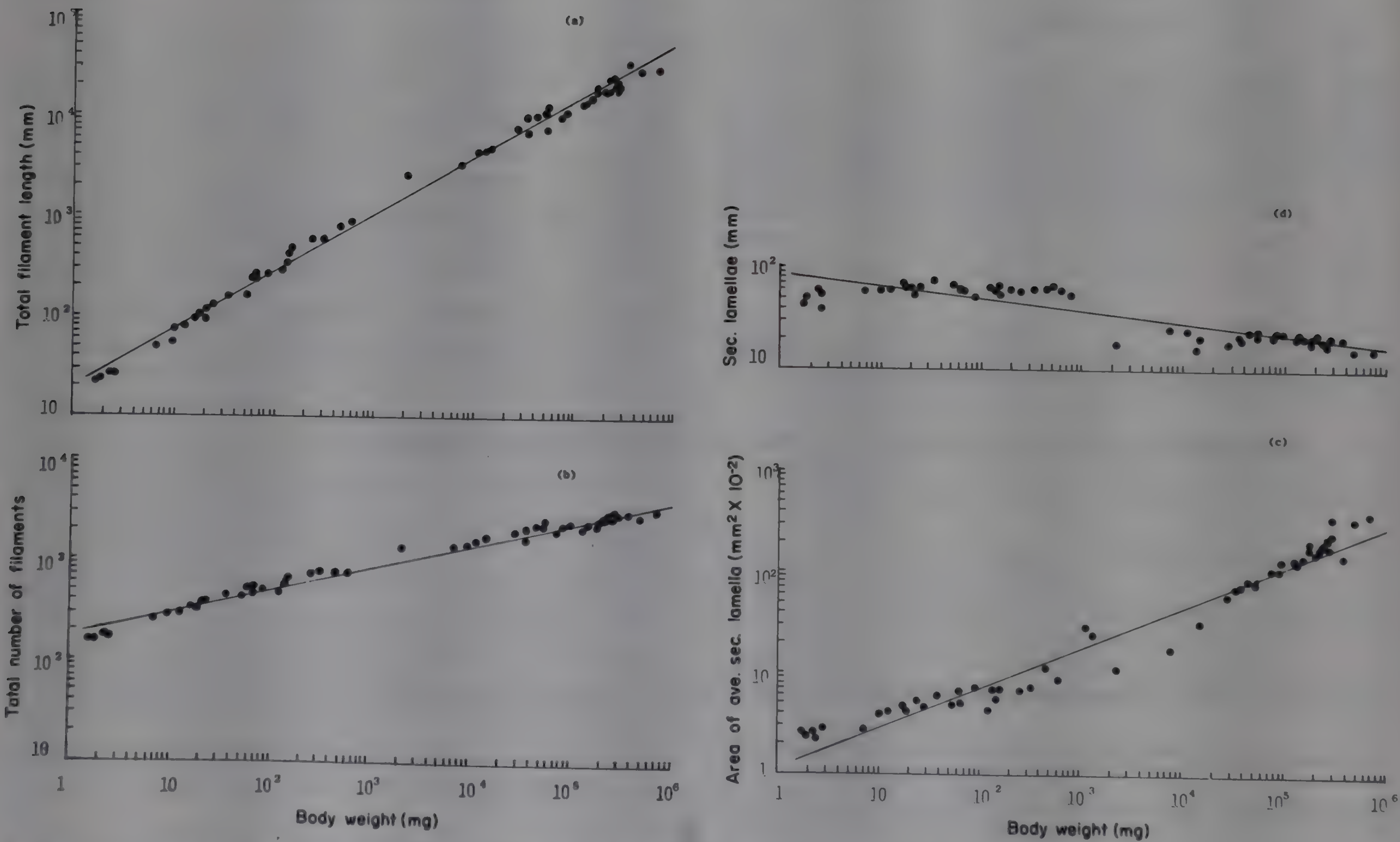

Fig. 3. Relationship between (a) total filament length, (b) total filament number, (c) area of an unweighted average secondary lamella and (d) secondary lamellae/mm of filament with weight.

Morphological Development of the Swimming and Feeding Apparatus in Larval Rabbitfish, *Siganus guttatus*[*]

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Abstract

The development of body parts for swimming and feeding in *Siganus guttatus* larvae was studied in samples reared in the laboratory at temperatures of 27.3-30°C. The dorsal and ventral fin rays began to develop at 3.93 mm total length (TL). The relationships TL vs. the greatest body depth and TL vs. position of the greatest body depth changed slopes at 5.50 and 5.90 mm TL, respectively. Flexion of the notochord was completed at about 6.50 mm TL. The adult complement of fin rays was developed by about 8 mm TL. As for the feeding-related parts, the premaxilla appeared at 4.12 mm TL and came to occupy most of the gape as in adults at about 6.5 mm TL. Jaw and pharyngeal teeth started to develop at 4-4.5 mm TL. From these observations, the larval stage of *S. guttatus* may be divided into three phases: (1) inactive swimming and feeding by swallowing (to about 4-5 mm TL); (2) the transitional phase (to about 7-8 mm TL); and (3) active swimming and feeding (8 mm TL and beyond). A change in feeding habits may be expected in *S. guttatus* larvae at sizes 7-8 mm TL as shown by this study.

Introduction

The purpose of this study is to describe the development of body parts related to swimming and feeding in larval rabbitfish, *Siganus guttatus*. This species is found in diverse habitats ranging from estuarine waters to coral reefs in the tropical and subtropical Indo-Western Pacific. Its high commercial value in Southeast Asian countries has encouraged experimental rearing of the species, fry having been produced at the Aquaculture Department of the Southeast Asian Fisheries Development Center (SEAFDEC), in Iloilo, Philippines since 1981. However, very little is known of the early life history of the species, especially of its morphological development.

Materials and Methods

Larvae for this study came from eggs spontaneously spawned on 1 April 1985. The facilities and methods for rearing have been described by Hara et al. (in press). Larvae were sampled daily from day 0 (hatching day) to day 21. A total of 174 larvae (1.94-12.18 mm TL) were sampled. They were fixed in 5% formalin and transferred to 70% alcohol. Observations were made on the fin rays, widest body depth and its position from the snout, and angle of notochord end. Ninety-nine specimens (2.52-10.99 mm TL, days 1-19) were cleared and stained following the method of Dingerkus and Uhler (1977). These specimens were used for observations on fin supports, vertebrae, jaw and pharyngeal teeth, and gape and premaxilla length. The methods of measurements and counts followed Kohno et al. (1983, 1984), and terminologies followed Kohno and Taki (1983). In this study, TL is given in the fresh state by using the following equations; TL (fresh) = 0.426 + 1.002 TL (preserved), and TL (fresh) = 0.320 + 0.979 TL (cleared and stained).

Results

The vertical fins were in the form of a membraneous finfold in the early larval stage. The anterior edge of both the dorsal and ventral finfolds receded as larvae grew and settled in place by 4.72 and 6.44 mm TL, respectively. The finfold separated into dorsal, anal and caudal fins at 6.94 mm TL.

The adult complement of the dorsal fin supports is 21, 11 for spines and 10 for soft rays (Fig. 1A), of which the most anterior pterygiophore supports three spines. The most anterior fin support first appeared at 3.70 mm TL. The development of the fin supports for spines proceeded posteriorly with growth, but the fin supports for soft rays appeared suddenly at about 5 mm TL. The adult complement gained about 6.35 mm TL. The first ossification was found in the most anterior fin support of a 4.96 mm TL specimen and the largest specimen examined, 10.99 mm TL, while nine fin supports for soft rays remained to be ossified.

The first visible dorsal fin ray appeared at 3.93 mm TL and became the second spine (Fig. 1A). The development mode of the fin rays was similar to that of the fin supports. The smallest specimen possessing the adult complement, 13 spines and 10 soft rays, was 8.04 mm TL.

On the other hand, 10 anal fin supports were first observed at 5.10 mm TL, and the adult complement of 14 fin supports was evident at 6.03 mm TL (Fig. 1B). The most anterior fin support commenced ossification at 7.17 mm TL, while in a 10.99 mm TL specimen the six anterior fin supports were ossifying.

The smallest specimen possessing the anal fin rays was 5.94 mm TL, with the most anterior three spines (Fig. 1B). The development mode of the anal fin rays was similar to that of the dorsal fin rays. The adult complement of seven spines and nine soft rays was first observed at 8.54 mm TL.

The development of the parhypural, hypurals and epurals, considered the main support of the caudal fin rays, was also traced in this study.

Hypural 1 of a 4.04 mm TL specimen was the first visible caudal complex element. Hypural 2 appeared at 4.14 mm TL, parhypural at 4.18 mm TL, hypural 3 at 4.63 mm TL, central epural at 4.96 mm TL, hypural 4 at 5.05 mm TL, posterior epural at 5.10 mm TL and anterior epural at 5.14 mm TL. At 6.03 mm TL, the hypurals 1 and 2 started to be ossified. Ossification started in the hypural 3 at 6.68 mm TL and in both the parhypural and hypural 4 at 7.17 mm TL. Hypural 5 appeared late at 8.94 mm TL. No ossification was observed in the epurals and hypural 5 of the specimens examined.

The first caudal fin rays to appear were six (3 + 3) principal rays in a 4.83 mm TL specimen (Fig. 1C). The number of principal fin rays increased as larvae grew, and the adult complement (9 + 8) was acquired at 6.44 mm TL. Thereafter, the secondary caudal fin rays were added on both the upper and lower lobes and developed fully at about 8.50 mm TL.

The development mode of the pectoral fin elements in *Siganus guttatus* is similar to those in other species (Kohno et al. 1983, 1984; Taki et al. 1986).

The cleithrum, coraco-scapular cartilage and blade-like cartilage, which later grew into actinosts 1-4, were present in a 2.52 mm TL specimen, the smallest examined. The development of the pectoral fin supports proceeded slowly, and the last elements to appear were actinosts 3 and 4 in a 10.80 mm TL specimen.

Pectoral fin rays developed from the blade-like finfold. The smallest specimen possessing pectoral fin rays was 6.29 mm TL, in which three rays were observed on the dorsal side of the finfold (Fig. 1D). Fin rays grew from the dorsal to ventral part of the finfold. The adult complement of 15-17 fin rays was first completed at 9.74 mm TL.

The ventral fin support first appeared at 3.70 mm TL, and ossification started at 4.18 mm TL. The first ventral fin ray to develop was the outer spine in a 3.93 mm TL specimen (Fig. 1D). The fin rays were added from outside to inside and were fully developed at 7.44 mm TL.

The first vertebrae observed were the most anterior two in a 6.29 mm TL specimen. Its formation went on posteriorly, the smallest specimen (7.17 mm TL) acquiring a fully developed vertebral column.

Notochord flexion began at 4.97 mm TL (Fig. 1E). Full flexion, with the notochord end at an angle of 40-50 degrees, was acquired at around 6.50 mm TL.

Based on the log-log plots, a flexion point was shown at about 5.50 and 5.90 mm TL in the widest body depth and its position related to the snout, respectively (Fig. 1F).

The first upper jaw tooth was found at 4.49 mm TL (Fig. 2A). The number of teeth increased gradually with growth until 7.50 mm TL when a rapid increase was observed. A lower jaw tooth was, on the other hand, first evident at 4.12 mm TL (Fig. 2A). The number of teeth increased from one beginning at 5.94 mm TL, and gradually thereafter.

An upper pharyngeal tooth first appeared at 3.26 mm TL, the number increasing after the larvae attained about 6.00 mm TL (Fig. 2B). On the other hand, the smallest specimen possessing lower pharyngeal teeth was 7.08 mm TL, in which two teeth were observed (Fig. 2B). The number of teeth increased gradually with growth.

The maxilla was first observed at 2.52 mm TL and occupied the gape entirely. The premaxilla was first seen at 4.12 mm TL and occupied 9.1% of the gape (Fig. 2C). The ratio of the premaxilla length to the gape increased rapidly with growth up to about 6.50 mm TL, after which it remained steady between 60 and 80%.

Discussion

The development of body parts for swimming and feeding in larval *Siganus guttatus* schematically represented in Fig. 3, needs elucidation.

The finfold functions as an important apparatus for swimming in the early larval stage; the vertical finfold extends a body dorso-ventralwards to add to the amount of surface pressed against the water (Gosline 1971), and the pectoral finfold allows more flexibility in movement (Blaxter 1969). Our observations indicate that *Siganus guttatus* larvae in the finfold stage endeavor to keep their position against the current by using the vertical and pectoral finfolds. During this finfold stage, no tooth appears and larvae are considered to take food by swallowing.

The first fin rays to appear are the second dorsal and the outer ventral spines in a 3.93 mm TL specimen. These spines, considerably elongate and bearing serrations along their anterior margin, are generally considered to increase the specific gravity of larvae and to serve against predation (Moser 1981; Johnson and Keener 1984). Following close to the appearance of the dorsal and ventral fin rays are the caudal and pectoral fin rays. The adult complement of fin ray counts is attained at 8 mm TL. Prior to this, several developments related to swimming occur: flexion of the notochord end, which causes a shift in the direction of caudal fin rays and increases forward propulsive force, is completed at about 6.5 mm TL; a change in the greatest body depth and its position is observed at 5.5 and 5.9 mm TL, respectively; the vertical finfold is completely separated into dorsal, anal and caudal fins at 6.94 mm TL; and the vertebrae are fully developed at 7.17 mm TL. These observations indicate that the swimming ability may be improved at around 7-8 mm TL.

Between 4 and 5 and 7 and 8 mm TL, development of the body parts related to feeding occur: the teeth start to appear on the upper pharyngeal at 3.43 mm TL, on the lower jaw at 4.12 mm TL, on the upper jaw at 4.49 mm TL, and on the lower pharyngeal at 7.34 mm TL. The number of teeth increases with growth, the premaxilla appears at 4.12 mm TL, and the ratio of the premaxilla length to the gape comes to occupy 60-80% at about 6.50 mm TL. These developments indicate that a change in feeding mode occurs at around 7 mm TL.

To summarize, the functional development of swimming and feeding in *Siganus guttatus* larvae may be broken down into three phases (Fig. 3): (1) inactive swimming and feeding by swallowing (to about 4-5 mm TL); (2) the transitional phase, in which the formation and improvement of the various body parts take place (to about 7-8 mm TL); and (3) the active swimming and feeding (8 mm TL and beyond). Based on the present study, a change in feeding habits of *S. guttatus* may be expected at sizes 7-8 mm TL.

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*SEAFDEC Aquaculture Department Contribution No. 194

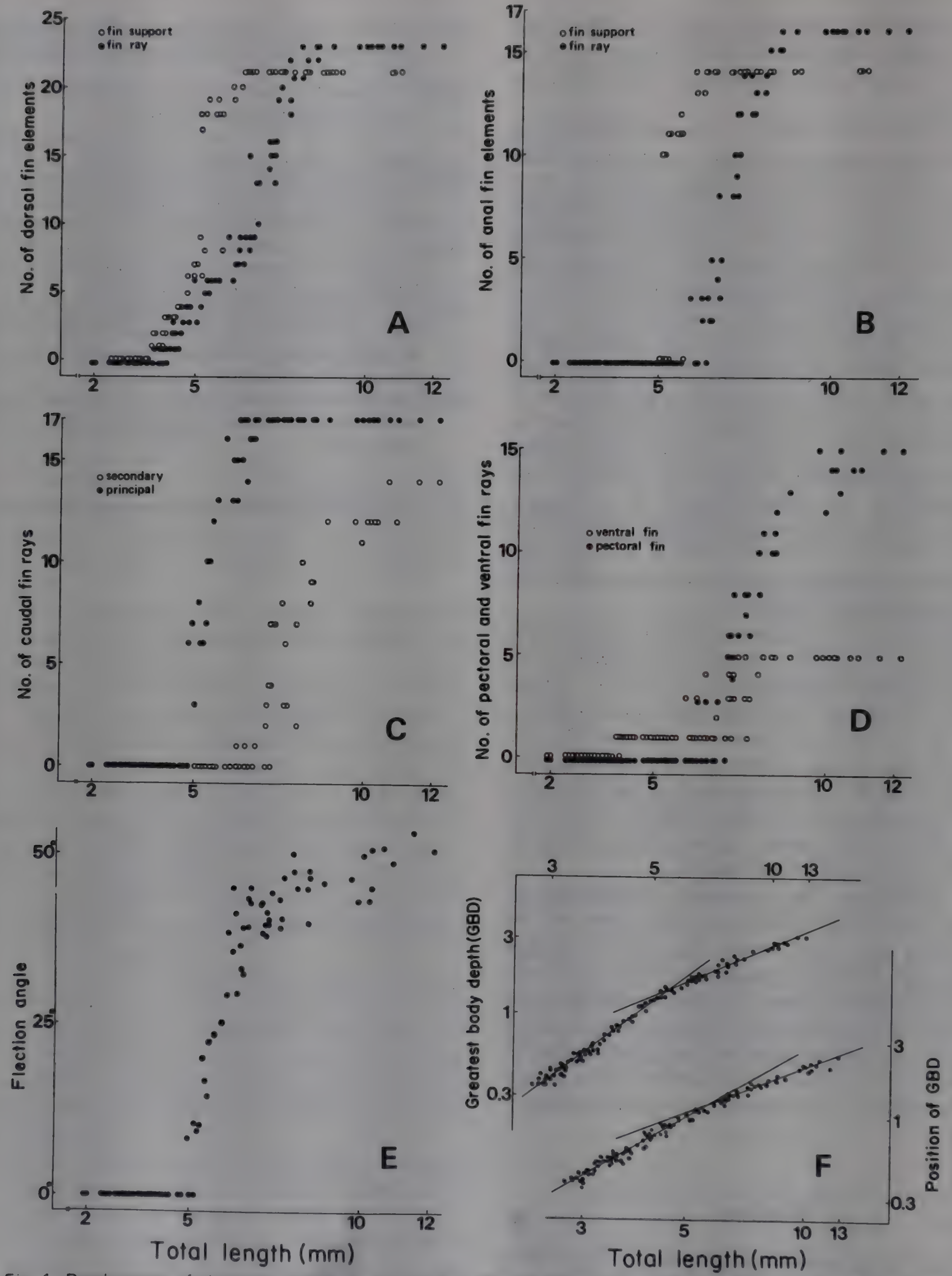

Fig. 1. Development of characters related to swimming in *Siganus guttatus* larvae. A: dorsal fin elements. B: anal fin elements. C: caudal fin-rays. D: pectoral and ventral fin-rays. E: flexion of notochord. F: log-log plots of the greatest body depth (GBD) and position of GBD.

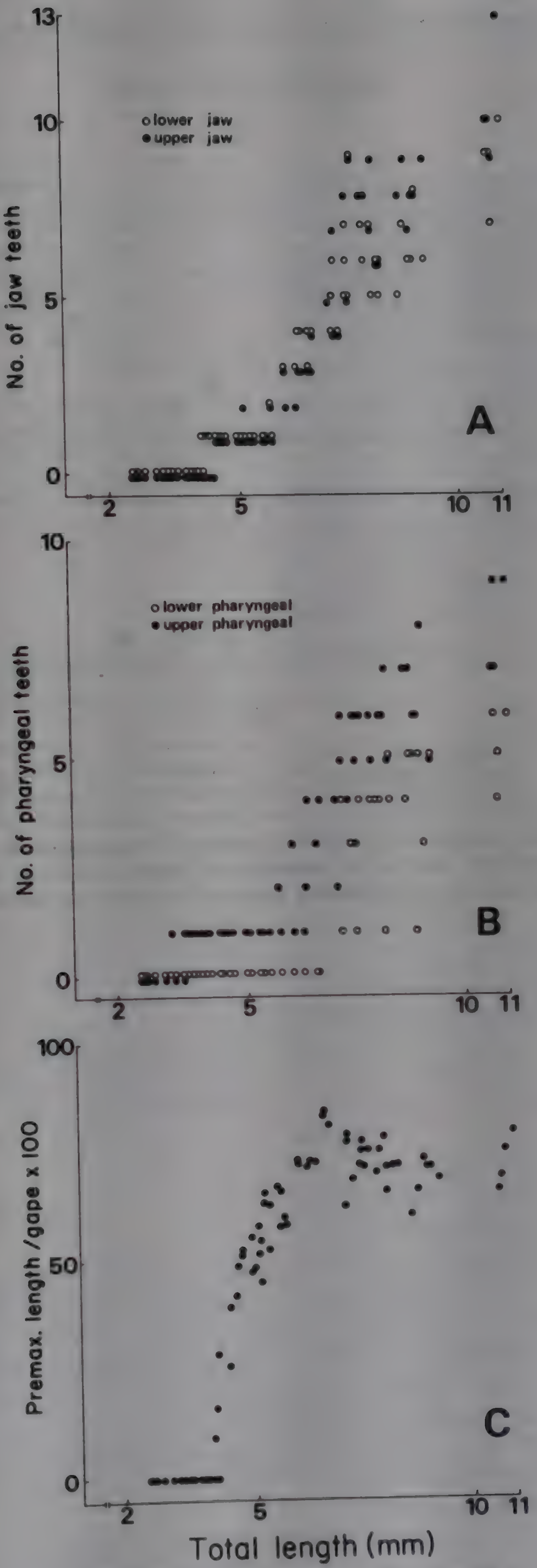

Fig. 2. Development of characters related to feeding in *Siganus guttatus* larvae. A: jaw teeth. B: pharyngeal teeth. C: ratio of premaxillary length to gape.

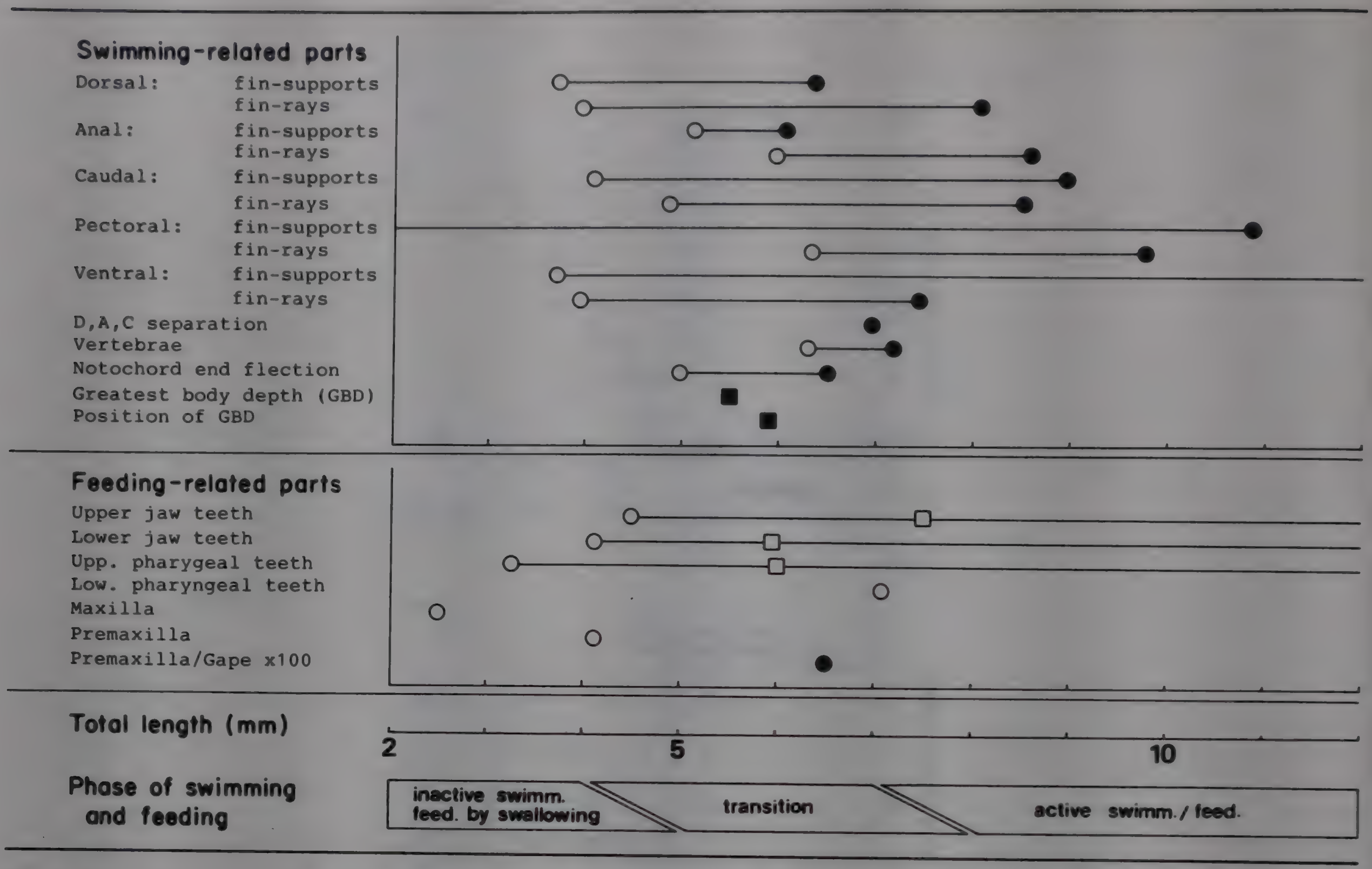

Fig. 3. Schematic representation of the development of characters concerned in swimming and feeding in *Siganus guttatus* larvae. Open circle, appearance of element or phenomenon; Solid circle, completion in number of element or in phenomenon; Open square, abrupt increase in number; Solid square, flexion point; D, A, C separation, separation of finfold into dorsal, anal and caudal fins.

Morphohistological Study of the Early Development Stages of the Red Sea Bream, *Pagrus major*

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Abstract

Facts on the early life of the red sea bream were supplemented and basic physiological changes common to marine finfishes during their metamorphic stage were clarified. Matured spawners were artificially bred, their eggs collected, incubated and reared to postlarval or prejuvenile size. A detailed morphohistological investigation on the early larval stages, particularly on the digestive system and accessory organs, was carried out. Hatching occurred at 40-44 hours after fertilization at 18-19°C. Ovulated eggs were buoyant and semitransparent with a centrally-located oil globule. A slight increase in egg diameter from 0.8 to 0.9 mm was noted after impregnation. The newly-hatched larvae, measuring 2.2 mm average length possessed a functional heart. Histological findings revealed early formation of the mouth furrow and the digestive tract. First feeding was observed on the fourth day after hatching and caudal ossification commenced on the tenth day. Larve in their yolksac stage, termed "protolarvae", were followed by the "mesolarval stage" until the whole caudal ossification was completed. The "metalarval stage" ended after the appearance of the pyloric appendages on the 30-day old larvae, just before it attained adult form. The size attained, not the age of the fish characterized the stage markers and the developed functional organs.

Introduction

The red sea bream, *Pagrus major* (Temminck and Schlegel), locally known as "Madai" in Japan where it is cultured intensively, is most widely distributed along the Pacific coast from the tropical to the subtropical region of Asia. This sparid fish has been the focus of significant research because of its high market value and popular demand, and for the improvement of its breeding, rearing, and other aspects of its culture, especially its early life stages.

The acquisition of fertilized eggs and mass culture of juvenile red sea bream was reported by Kitajima (1978), but no detailed published information is available on its embryology and early development. Previous histological studies on the teleost larvae generally dealt with the initial and succeeding stages of its feeding and the structure and function of the digestive tract, as was reported by Tanaka (1969a, 1969b, 1971).

The morphohistological function of the red sea bream was studied to describe the gross embryogeny and the morphohistological development of its early life stages.

The study was conducted at the Usa Marine Biological Research Institute of Kochi University in Japan in 1980-1981. Histochemical and other analytical works in the latter part of the study were conducted at the Laboratory of Aquatic Ecology, Department of Cultural Fisheries, Faculty of Agriculture, Kochi University, Nangoku City.

Materials and Methods

Breeding preparations were done a month before the expected spawning, which usually occurs from early April to May in the vicinity of Tosa Bay, Kochi Prefecture. Matured five- to seven-year old breeders of both sexes, 2-3 kg and 30-50 cm total length were acquired from commercial farms and transferred to a net cage of about a kilometer from the marine hatchery. Breeding, incubating and larval rearing tanks were thoroughly sterilized prior to transfer of breeders in preparation for the induced spawning. Preliminary sexing was done before the fish were released to the tank and individual spawners were tagged.

After a day of conditioning, a dose of gonadotropin (HCG 2,000 units/ml) 0.1 ml/1 kg body weight was injected intramuscularly. An additional dose of 500 to 600 units was administered depending on the effect of the initial treatment after 12 hours. The gravid fish were stripped and the eggs collected in a plastic bowl. The dry method of fertilization was employed using a bare finger to mix the sperm and eggs thoroughly. About 3-5 min. were then allowed to elapse to ensure a high rate of fertilization. Eggs were then placed in a fine-mesh net and washed in a bucket under gently running seawater.

Preliminary determination of fertilized eggs was done in a test tube or beaker filled with seawater, as good eggs tend to float and occupy the upper half of the container, while the unfertilized eggs settle at the bottom. The same procedure was used to measure the rate of fertilization by volumetric method. Final detection of fertilized eggs, however, was done randomly through microscopic analysis.

The fertilized eggs were finally transferred to 1-t fiberglass quadrangular tanks for the whole incubation period to hatching at a controlled temperature range of 18-19°C. The eggs were placed in 25-40 cm^3 suspended incubating nets aerated gently to avoid stress at constant seawater salinity range of 34.6-35.2 ppt during the whole incubation period. Embryonic development was monitored with a light stereo microscope recording and photographing the significant stages. Dead and sinking eggs were regularly siphoned off.

A day after hatching, the larvae were released from the incubating net enclosures at the same temperature and with the other controlling factors maintained. *Chlorella* was first introduced into the larval rearing tanks at least 3 times a day at densities 20-40 x 10^4 cells/ml. Live food was introduced on the 4th day after hatching, consisted of rotifers *(Brachionus plicatilis)* which were later combined with copepods *(Tigriopus japonicus)* after 20 days. At the later postlarval stage, the food was changed to non-living form consisting of mysid shrimps *(Euphausia pacifica)* mixed with a commercial fishmeal and sand lance *(Ammodytes personatus)*, all in minced form.

At this stage, regular change of tank water was done twice each day by a flow-through system which replenished at least 1/3 of the level of water in the culture tank. The surrounding area was also cleaned with a siphon. Aeration was slightly increased, corresponding to the growth of larvae, while physicochemical parameters in the the tank were continuously recorded. As the larvae grew older, a bigger tank was provided to allow more space for the fish and the feeding schedule was lessened to at least twice each day while the density of the food was gradually increased.

Larval sampling was done periodically to record the metamorphosis from hatching to postlarval stage. Photographs were taken at every significant stage and morphological observations done with a light stereo microscope. Samples taken randomly were preserved in 10% formalin after measurement then fixed in Bouin's solution and were sectioned into 6-10μm thickness with a microtome for the histological study. Staining procedures follow that of Sano (1965).

Prepared tissues were stained with Mayer's hematoxylin and counterstained with eosin. Other groups were stained with periodic acid Schiff's reagent and Giemsa's stain, while some were fixed with 4% osmium tetrachloride in Dalton's buffer and saccharose solutions. Fish in the later postlarval stage were cut into two parts and the abdominal region fixed and included in the histological preparations. All observations were done with a light microscope and histomicrographs of the serial sections were taken at every relevant stage.

Results and Discussion

The gonadotropic hormones produced positive results in the injected breeders within 12 hours. Stripped eggs were semitransparent, and could be barely seen by the naked eye as they floated along the water surface and subsurface. It was noted that newly fertilized eggs possessed coarse granulated cytoplasm with a distinct centrally located oil globule. Normal cleavage was observed four to five hours after fertilization, blastulation and gastrulation followed with the subsequent formation of the primordial body anlage. First body twitching was recorded at 35-39 hours after fertilization. Hatching occurred simultaneously at 40-44 hours after fertilization. Fig. 1 shows the embryonic development stages from fertilization to the prehatching stage.

Newly hatched larvae measured 2.2 mm average length and 0.6-0.7 mm body depth and had 26-28 somites. The oil globule was positioned posteriorly to the yolk mass along the anal region in front of the urinary vesicle. The day-old larvae had increased in length to 27 mm length. The mouth opened at day 3. Eye pigmentation was simultaneously visible with the pectoral fin bud. Initial feeding reactions were observed at day 4. Yolk sac was totally absorbed on day 6 and active feeding was noted. Caudal ossification started from days 10 to 16 and distinctive epural and hypural bones were differentiated on day 22-24. Dorsal and ventral fin-rays became more distinct at this stage while all the finfold borders along the body margins continuously diminished in appearance. On day 28-30, a full body resemblance to that of the adult form was completed with distinguishable cross bands along the lateral body margins, fully developed fins, and more prominent and functional eyes. The morphological development stages from day 0 to day 30 are illustrated and described in Fig. 2 and Table 1, respectively.

The protolarvae were histologically proved to possess a primitive gut at the hatching stage. The mouth anlage was still closed, forming a furrow in the head region between the optic lobes. Day-old larvae were observed to have a vertical layer of cells along the head region forming the presumptive gill archs. Kidney tubules were likewise evident along the urinary bladder. In day 2, liver-initiating cell formations were identified. Mid-gut and hind-gut constriction was apparently developed in 3-day old larvae. Gill arches were also distinct, simultaneously appearing with the gall bladder. Food particles were clearly stained in day 4 samples and primordial but functional digestive organs were detected. The last digestive accessory organ positively detected histologically in day 30 was the pyloric caeca appearing as an outgrowth of the anterior intestinal region near the junction of the pylorus. Significant results of the histological studies undertaken are summarized in Table 2.

The slight increase in diameter of unfertilized ova from 0.8 to 0.9 mm in the nearly fertilized egg was definitely due to sperm impregnation. The process was followed by the distinct appearance of a perivitelline space along the outermost chorionic membrane a few minutes after fertilization.

On the embryological aspect, the late phase of organogeny to the prehatching stage was the longest embryonic development phase, characterized by the formation of primordial organs such as the Kupffer's vesicle, optic lobe, cerebral region, body anlage, the first heart palpitations and body movement which might be common to pelagic fish embryos in their ontogenic stage.

It was remarkable to note the pectoral fin bud and the eye cup to be the first external organs morphologically differentiated at this very early stage. This could be a provision to enable the fish to establish balance. The same organs were later observed to have a vital function in initial feeding activities when prominent retinal pigmentation and functional pectoral fins were being developed.

Primitive epithelial cells lining the digestive tract from the buccal region to the anal epithelium included different types of cells with special functions in digestion -- taste buds and other receptor cells, goblet cells, mucus cells and the numerous fat vacuoules lining the postintestinal region where fat droplets were positively stained by the osmium reagent.

In this study, complete metamorphosis was recorded upon detection of the pyloric appendages on day 30 when the fish fully resembled the adult. In the red sea bream, marks that characterize the embryonic and larval stages are mainly due to the advanced development of functional organs and by the size attained by the fish and are not precisely determined by its age.

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Table 1. Morphological differentiation of major organs and body structures of live red sea bream samples from hatching to early juvenile stage.

Age (days)	Head region	External and internal body region	Fins
0	av;h;oc	uv	pfb
1			
2	sf	ac	
3	lj;m;uj	ic;ig;pc	1*pfs
4	ep;ga	ao	
5		ag;fm	
6	np	bc;bf;bv;ls;oga;p;ya	
7	gf	ab;vs	
8	od	my	6*pfs
10	ob		
12	rgs		
13	ov		
14	rha	rbc	
15	4*ob	rsp	
16			cb;dmf;vmf
18			cfr;hy
21			7+9*dfr;2*cfs
23	tj		10*afr;10+11*dfr;plf**
24			ffa
25	2*np		4*cfs;hcf
26			6*cfs
27			11*afr;10+12*dfr
28		ll;sc	
32		6*lcb	9*cfs
36			plfs
40			10*cfs

*Number of fin rays or segmentations and meristic counts.

**Structures that might have been developed earlier.

Legends.

Head region: av, auditory vesicle; ep, eye pigmentation; ga, gill archs; gf, gill filaments; h, heart; lj, lower jaw; m, mouth; np, nasal pores; ob, opercular blades; oc, optic cup; od, otolith disappearance; ov, oral valve; rha, reddish heart appearance; rgs, reddish gill structure; sf, snout formation; tj, teeth on jaws; uj, upper jaw.

External and internal body region: ab, air bladder; ac, alimentary canal; ag, anal groove; ao, anal opening; bc, blood cells; bf, blood flow; bv, blood vessels; fm, food materials; ic, intestinal convolution; ig, intestinal groove; lcb, lateral cross bands; ll, lateral line; ls, liver(-like) structure; my, myomere; oga, oil globule absorption; p, peristalsis; pc, post constriction; rbc, red blood cells; rsp, reddish spleen; sc, scales; uv, urinary vesicle; vs, vertebral spine; ya, yolk absorption.

Fins: afr, anal fin rays; cb, caudal bending; cfr, caudal finrays; cfs, caudal fin segments; dfr, dorsal fin rays; dmf, dorsal marginal fin; ffa, finfold absorption; hcf, homocercal caudal formation; hy, hypural; pfb, pectoral fin bud; pfs, pectoral fin segments; plf, pelvic fin; plfs, pelvic fin segments; vmf, ventral marginal fin.

Table 2. Histological differentiation of major digestive organs and accessory parts from hatching to metalarval stage.

	Organs							
Age (days)	Buccu pharyngeal	Esophagus	Stomach	Intestine	Rectum	Pancreas	Liver	Others
0	ma							h
1	ga							k
2				ic	rc		hc	gb
3	go;lj;mo;uj			cc		lc;pa;zg	bd	ab
4	ov		pv		ub*			bc
5		lf	cc	m	ao			bv
6		mc			vc			
7								
8				od		pd		
9			lf					
10								
11								
12	pt;tb							sp
13				vc				
14								
15							hp	
16							gl	
17								
18								
19				gc				
20								
21	tj		bs				lv	
22			gg					
23								
24	mc		zg		gc			
25								
26								
27								
28					as*			
29								
30				pc				fa

*Organs that might have been morphologically developed earlier.

Legends:

Buccu-pharyngeal: ga, gill archs; go, opening; lj, lower jaw; ma, mouth anlage; mc, mucous cells; mo, mouth opening; ov, oral valve; pt, pharyngeal teeth; tb, taste bud; tj, teeth on jaws; up, upper jaw.

Esophagus: lf, longitudinal folds; mc, mucous cells.

Stomach: bs, blind sac; cc, columnar cells; gg, gastric glands; lf, longitudinal folds; pv, pyloric valve; zg, zymogen granules.

Intestine: cc, columnar cells; gc, goblet cells; ic, intestinal convolution; m, microvilli; od, oil droplets; pc, pyloric caeca; vc, vacuole cells.

Rectum: ao, anal opening; as, anal sphincter; gc, goblet cells; rc, rectal cells; ub, urinary bladder; vc, vacuole cells.

Pancreas: lc, Langerhans cells; pa, pancreatic cells; pd, pancreatic duct; zg, zymogen granules.

Liver: bd, bile duct; gl, glycogen granules; hc, hepatic cells; hp, hepato-pancreatic duct; lv, liver vacuoles.

Others: ab, air bladder; bc, blood cells; bv, blood vessels; fa, fat accumulations; gb, gall bladder; h, heart; k, kidney; sp, spleen.

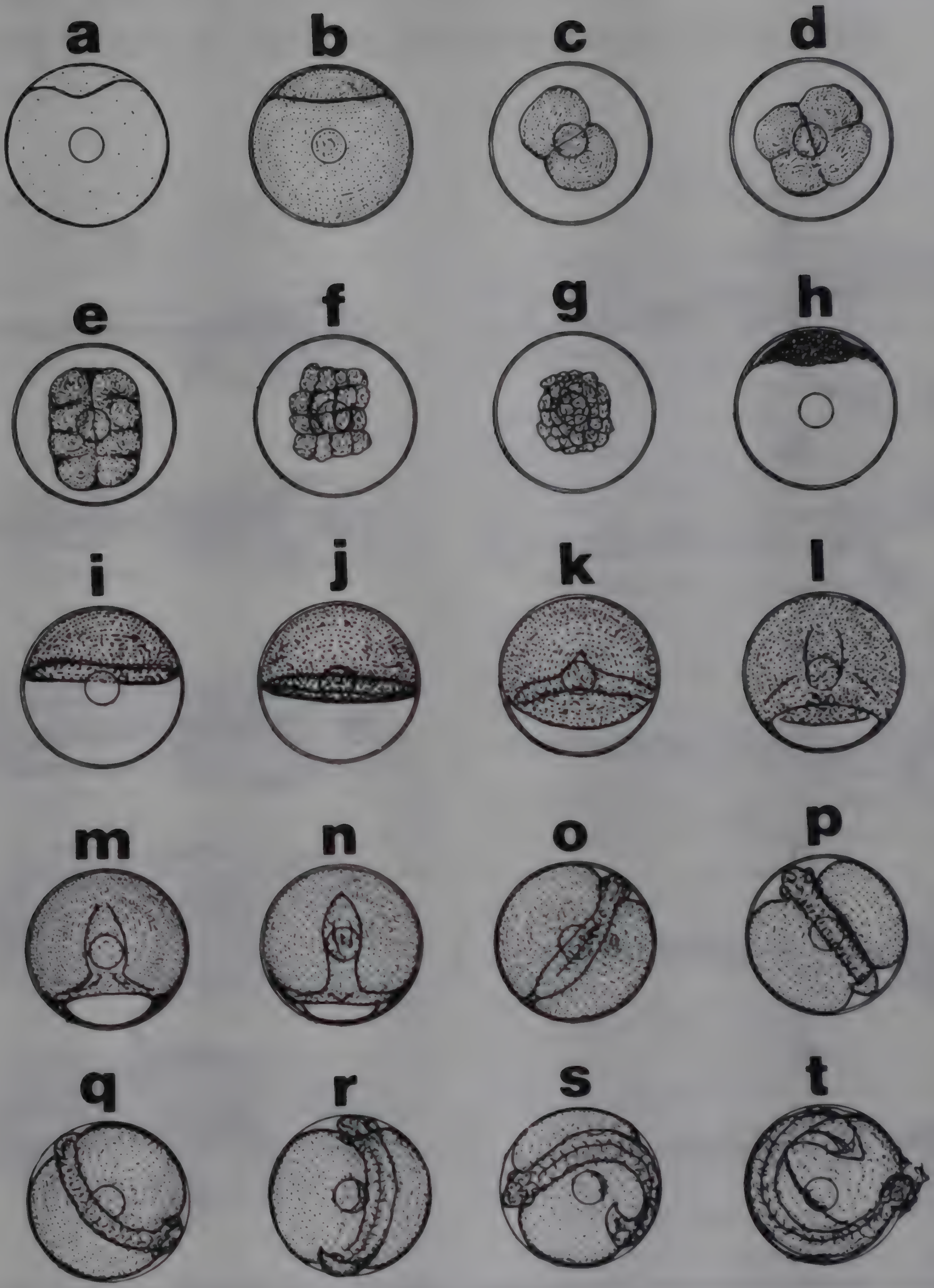

Fig. 1. Schematic illustration of the eggs and embryonic stages in the red sea bream from fertilization to pre-hatching. a = unfertilized zygote; b = newly fertilized egg; c-g = cleavage; h-j = blastulation; k-m = gastrulation; and n-t = organogeny.

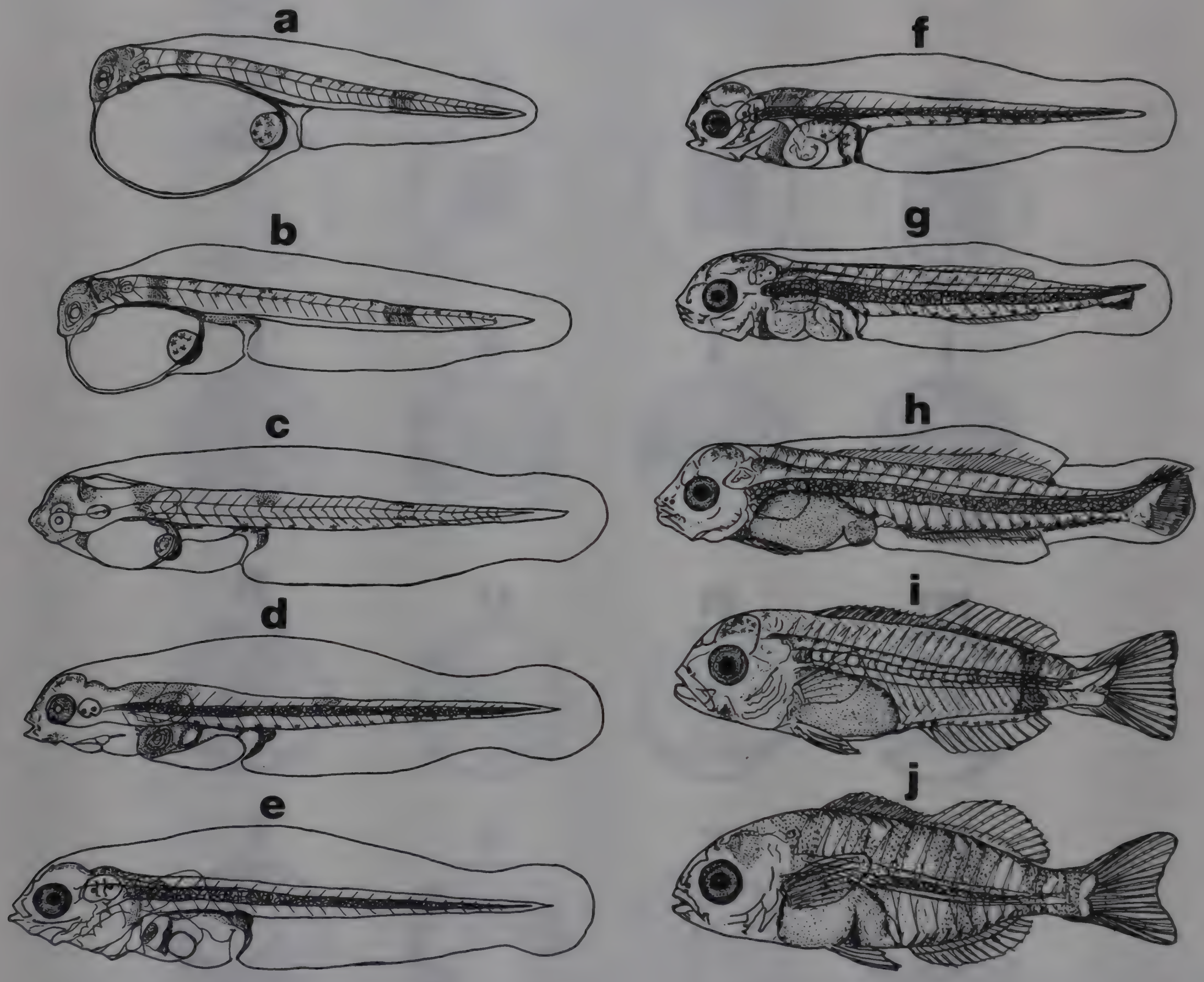

Fig. 2. Schematic illustration of the early larval developmental stages in the red sea bream from hatching to the metalarval stage. A = newly hatched larva, TL 2.2 mm; B = 1-day-old larva, TL 2.7 mm; C = 2-days-old larva, TL 3.0 mm; D = 3-days-old larva, TL 3.2 mm; E = 4-5 days-old larva, TL 3.1 mm; F = 6-7 days-old larva, TL 3.2 mm; G = 10-16 days-old larva, TL 4.0 mm; H = 18-20 days-old larva, TL 6.0 mm; I = 22-24 days-old larva, TL 6.5 mm; and J = 28-30 days-old larva, TL 13.0 mm.

Larval Rearing of *Macrobrachium rosenbergii* (de Man) in Brine Solution and Sea Salt

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Abstract

To evaluate the possibility of utilizing brine solution and sea salt in the larviculture of *Macrobrachium rosenbergii* (de Man) three treatments were used: combination of brine solution, de-ionized freshwater and *Chlorella* culture (greenwater) T_1; combination of sea salt, de-ionized freshwater and greenwater (T_2); and combination of seawater, de-ionized freshwater and greenwater (T_3). Salinity was maintained at 12-14 ppt.

Results showed that T_1 had the highest survival rate of postlarvae at 25.7%, followed by T_3 with 17.95% and T_2 with 6.71%. Mean percentage survival differences between T_1 and T_2 and between T_2 and T_3 were significant ($P < 0.05$). No significant difference was detected between means of T_1 and T_3.

The study indicated that brine solution can be effectively used in the larviculture of the giant prawn. It also showed the possibility of economically rearing prawn larvae in brackishwater reconstituted from sea salt.

Introduction

Nowadays, giant freshwater prawn *(Macrobrachium rosenbergii)* larvae are reared with the conventional techniques where artificial brackishwater of about 12 ppt salinity is prepared from seawater. It is very difficult to rear prawn larvae in remote areas far from the sea which entails added cost of getting seawater from its source to the inland hatcheries (Tansakul and Limpadanai 1980). Hauling of seawater requires a large vehicle and container; otherwise, frequent hauling of the same must be done. The utilization of more concentrated brine solution and sea salt in the larviculture of the giant prawn has so far received very little attention from aquaculturists.

Materials and Methods

This study was conducted from September 1983 to February 1984 at the wet laboratory of the Freshwater Aquaculture Center (FAC), Central Luzon State University, Nueva Ecija, Philippines.

Brine solution (165 ppt salinity) and sea salt were obtained from salt producers. Seawater was hauled from Lingayen Gulf along the sea coast of San Fabian, Pangasinan. The total hardness of the freshwater coming from the well of the Center was lowered from 240 to 166 mg/l $CaCo_3$ with a water de-ionizer.

Berried females of *M. rosenbergii* seined from the FAC ponds were reared in aquaria for larval production. When eggs were about to hatch, seawater was gradually added to increase the salinity of the rearing medium to about 5 ppt. Larvae produced were siphoned off and acclimatized gradually to a salinity of 12 ppt.

Three treatments with three replicates each were used. The treatments were as follows: T_1-combination of brine solution, de-ionized freshwater and greenwater; T_2-combination of sea salt, de-ionized freshwater and greenwater; and T_3-combination of seawater, de-ionized freshwater and greenwater (control). Each rearing medium was prepared by diluting brine solution, saltwater or seawater with de-ionized freshwater until the salinity fell within the desirable range of 12 to 14 ppt. Greenwater from an outdoor *Chlorella* culture was added at a ratio of about 1:25 parts greenwater to rearing medium.

The study was divided into two batches as only six tanks were available. Experimental treatments were set in a randomized block design.

Six circular conical-bottomed 250-l fiberglass tanks were filled with the rearing medium three days prior to stocking. The two airlift pipes incorporated in the design of the tank (Fig. 1) were used to provide a circulating airlift aeration system.

The number of larvae stocked in each tank (Table 1) was estimated by the aliquot method. Brine shrimp nauplii (BSN) and egg custard were utilized as feeds. The quantity of BSN given daily (expressed in teaspoon (tsp) of artemia cysts) from day 1 to the end of the rearing period was about 1/2 tsp/10,000 larvae.

Starting on day 10, egg custard (particle size approximately 0.3-mm diameter) was given twice a day, except Sundays, between BSN feeding, at about 10 a.m. and 1 p.m. and in excess to ensure that all larvae were able to grasp food particles.

Tank bottoms were cleaned at least once a day. Every other day starting day 5, about 5% of the total water volume was siphoned out and gradually replaced with an equal amount of fresh medium. Water level was maintained at 250 l.

Larval rearing was terminated when about 90-95% of the prawns had metamorphosed to postlarvae which were counted and transferred to holding tanks. Those that had not metamorphosed were further reared in basins containing their medium.

Water quality parameters of each rearing medium such as temperature, dissolved oxygen, pH and ammonia were recorded. The total hardness of each rearing medium and that of the de-ionized freshwater were also determined. The salinity of the rearing medium was checked once a week with a refractometer.

Results and Discussion

Survival rate of T_1 was the highest (25.74%), followed by T_3 with 17.95% and T_2 with 6.71% (Table 2). Differences between T_1 and T_2 and between T_2 and T_3 were significant ($P < 0.05$). Replicates within the same treatment differed in survival rates as found also by Ong (1975) and Aniello and Singh (1980).

Larval growth period to first day of metamorphosis was variable (Table 3). T_1 had the earliest mean at day 28.33, followed by T_3 and T_2 at days 30 and 32.33, respectively. The table also shows that T_1 had the shortest mean larval cycle of 43 days, followed by T_3 with 46.33 days the longest was that of T_2 with 61.67 days.

The larval cycle in T_1R_1 was completed during the warm period of the year. Part of T_2R_1, T_3R_1, T_1R_2, T_2R_2 and T_3R_2 experiments and the whole of block 3 experiments (i.e., T_1R_3, T_2R_3, and T_3R_3) were conducted during the cold months of the year (Table 1). Rearing experiments conducted during the cool periods had longer larval cycles. This agrees with the findings of Gibson (1975), Suharto et al. (1980) and New and Singholka (1982) that at temperatures lower than optimum, below 24-26°C, the larval rearing time is lengthened.

The differences in the mortality rates among treatments may be due to the differences in the mineral compositions of the rearing media used. Other possible causes of larval mortalities include: (a) poor water quality, i.e., higher NH_3-N in other tanks; (b) jumping larvae that get stranded along tank wall above the water line; (c) cannibalism; and (d) improper acclimatization prior to stocking.

Mass mortalities of the larval stock were observed during the mid-period of T_1R_1 experiment. The larval population, however, recovered after two weeks of frequent water exchange. Larval mortalities were suspected to have been caused by poor water quality. Ammonia concentrations in the rearing medium during the period when mass mortalities occurred were at the highest levels at 0.42 and 0.37 ppm.

Larval mortalities attributed to jumping larvae that got stranded along the tank wall were minimal. Larvae were noted to start jumping and as early as day 20. Increases in larval mortality from jumping occurred during power failures when the aeration system became inoperative. Power failures occurred during T_1R_1, T_1R_3 and T_2R_3 experiments.

Mortalities due to cannibalism (Peebles 1978). Aquacop (1977) and Suharto et al. (1980) also occurred, most seriously when the postlarvae appeared.

Improper acclimatization prior to stocking was also suspected to be one cause of larval mortalities in T_2 experiments. In this treatment a substantial number of larvae died within five days after stocking.

Conclusions and Recommendations

Results of the study indicate that brine solution can be effectively used in the larviculture of the giant prawn. There is the possibility, however, of economically rearing prawn larvae in brackishwater reconstituted from sea salt, but this method needs further investigation and improvement.

Further studies are recommended to improve the use of brine solution and sea salt in the larval rearing of the giant prawn, i.e., longer acclimatization. The highest concentration of brine solution to be hauled from salterns that will give comparatively good results should also be determined. Causes of poor survival in brackishwater made from sea salt should be investigated and hatching of prawn eggs in larval rearing tanks should be tried to minimize larval mortalities in the stocking process.

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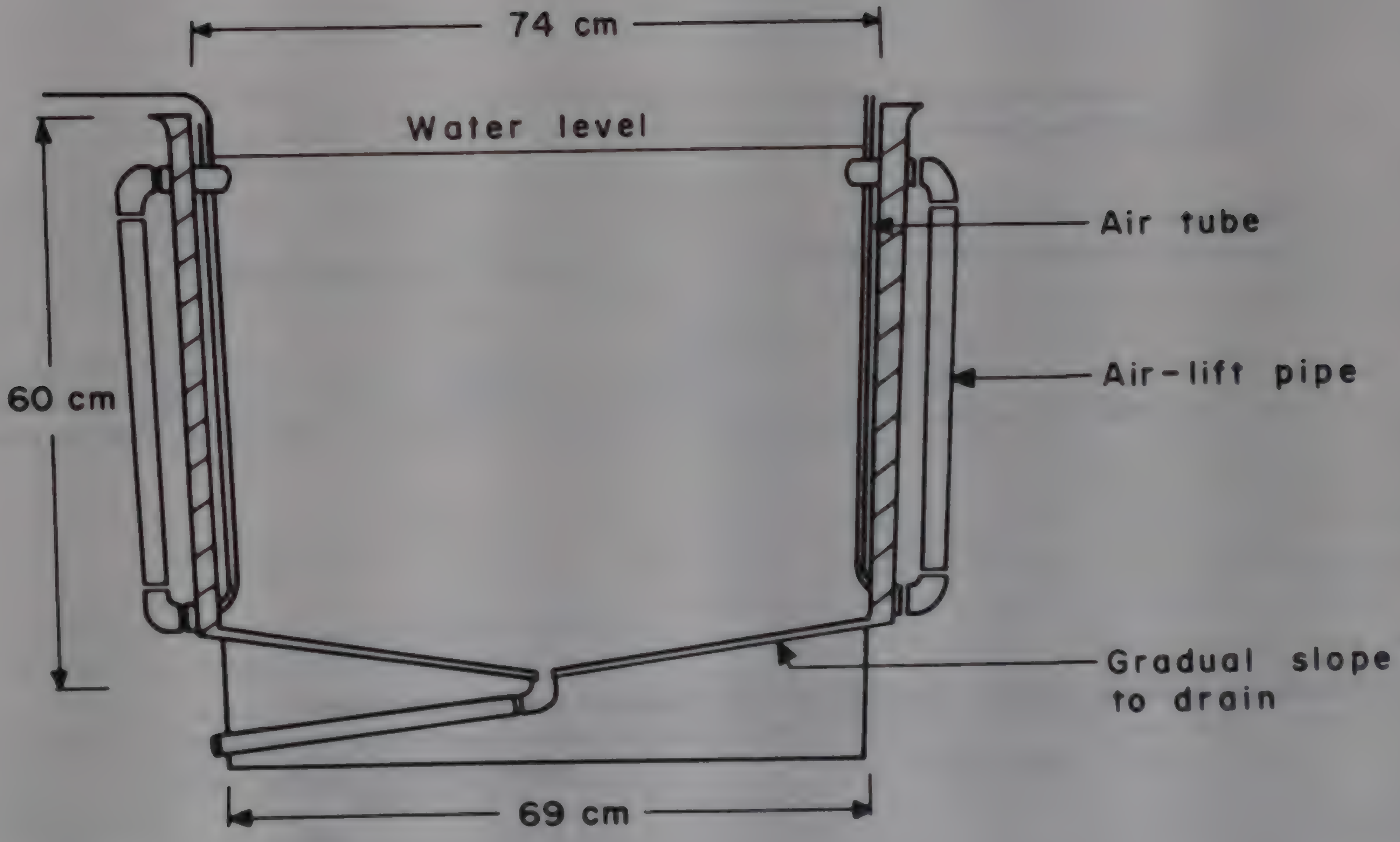

Fig. 1. A 250-l fiberglass rearing tank.

Table 1. Dates of hatching and stocking, estimated larvae stocked and stocking rates/l. T_1 = treatment one; R_1 = rearing trial one, etc.

	Date			
	Hatching	Stocking	No. of larvae/tank	No. of larvae/l
T_1R_1	Sept 27	Sept 29	11,378	45.5
T_2R_1	Oct 17	Oct 19	9,269	37.1
T_3R_1	Oct 12	Oct 14	11,470	45.9
T_1R_2	Oct 15	Oct 17	11,222	44.9
T_2R_2	Oct 19	Oct 22	8,060	32.2
T_3R_2	Oct 16	Oct 18	10,109	40.4
T_1R_3	Nov 10	Nov 12	10,891	43.6
T_2R_3	Dec 18	Dec 20	9,466	37.9
T_3R_3	Dec 21	Dec 22	10,186	40.7

Table 2. Percentage survival of *M. rosenbergii* from larvae to postlarvae.

Treatment	Survival (%)			Treatment mean (%)
	R_1	R_2	R_3	
1	28.85	20.61	27.76	25.74
2	2.88	5.76	11.48	6.71
3	15.44	18.14	20.26	17.95

Table 3. Metamorphosis time from larval to postlarval stage of *M. rosenbergii* in various rearing trials.

Treatment	Metamorphosis (no. of days)			Mean of metamorphosis (no. of days)		Average (no. of days)
	R_1	R_2	R_3	First	Last	
1	26-39	29-45	30-45	28.33	43.00	35.66
2	32-47	33-48	32-60	32.23	61.67	42.00
3	30-48	30-46	30-45	30.00	46.33	38.16

Ecology and Biology of Giant Mantis Shrimp, *Lysiosquilla maculata* Fabricius in the Philippines

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Abstract

This ongoing research deals with the distribution and abundance of giant mantis shrimp, *Lysiosquilla maculata*, in Polo Pt., Plaridel, Misamis Occidental, and Sitangkai Tawi-Tawi Islands, Philippines. A comparative survey gave a clumped or aggregated type of distribution for both stations at a density of 3.56 shrimp/ha and 4.2 shrimp/ha, respectively.

The nature of bottom sediments appears to be an important factor in the distribution of mantis shrimps. They generally prefer fine coral-sand substrates and very fine sediments.

Introduction

Mantis shrimps are a delicacy in Hawaii, Japan and other Asian countries (PCARR 1981). In the Philippines, they are not common except in parts of Mindanao. The present research aims to provide baseline information on the ecology and biology of the giant mantis shrimp, *Lysiosquilla maculata*.

L. maculata, locally known as "kamun" (Tausugs) or "amuntaha" (Cebuano), is a predatory crustacean (Caldwell and Dingle 1976). It is known to be the largest species of the order Stomatopoda, the most common and the most widely distributed species of the genus *Lysiosquilla* in the Indo-West Pacific region (Manning 1978). This species thrives in shallow waters of the intertidal zone toward the reef breaker zone characterized by fine coral-sand substrates.

Materials and Methods

The study was undertaken in Polo Pt., Plaridel, Misamis Occidental tidal flats (Fig. 1). The area is about 990,000 m^2, approximately 3.7 km east southeast of Looc wharf, Plaridel.

Field surveys were made in neighboring reef areas of northern Mindanao and in Tawi-Tawi at the southern tip of Mindanao, which is noted to be a good source of mantis shrimps.

Monthly field sampling was done over eight months during the lowest tide for easy location of mantis shrimp burrows, using the modified systematic distance method (Westmann 1971) cited in Loya (1978). Characteristics of mantis shrimp burrows and other ecological parameters were investigated. A market survey was also conducted.

Results and Discussion

L. maculata thrives in shallow waters and is found in selected neighboring reef areas of Baliangao and Lopez-Jaena, Misamis Occidental. It is also common all around Sitangkai and Sibutu Islands of Tawi-Tawi. In the main study area at Polo Pt., Plaridel, about 16 ha were surveyed. Fifty-seven shrimps were observed from 46 identified burrows at a density of 3.56/ha (Table 1). In Sitangkai Islands, 10 ha were covered during sampling, for a density of 4.2 shrimps/ha (Table 2). The collection of shrimps was limited by the inefficiency of the traps and the number of available expert collectors, although many burrows were located. Both stations exhibited aggregated or clumped distribution. The species occurred in the study areas throughout the study period.

Habitat. Mantis shrimp burrows are found from the intertidal down to the reef breaker zone. They are characterized by either a volcano-like mound of sediments or a funnel-shape crater. The burrows show a J or L shape with openings proportional to the animal's size. The most frequent burrow diameter was 68-73 mm with shrimps having a carapace length of 32-49 mm (male) and 34-50 mm (female) (Table 2). Each burrow was usually inhabited by a pair of mantis shrimps, male and female. In the inner burrow wall, the sediment is held together by a sticky substance which is probably excreted by the mantis shrimp. Burrow openings are difficult to distinguish from other animal burrows found in the intertidal flat because they are concealed with very fine sediments. During daytime, the eyes of the shrimps are seen through a tiny hole from their concealment. At night, the hole is relatively large.

Collection. Collection of mantis shrimps was usually done during low tide. Burrows were identified with the help of local fishermen in the area. The fish trap, locally called "lit-ag", was baited with various fish. A baited stick with a rattan loop is fitted to the opening of the shrimp burrow, while the other end is tied to a long bamboo pole. This is made to rest on another bent bamboo twig with a sort of trigger held by a string, being pulled by the long pole. A slight pull of the bait triggers the trap, thus closing the loop and consequently hanging the shrimp together with the baited stick.

The adult female is usually distinguished from the male by its distinct tangerine color at the mid-dorsal abdomen, which indicates developing ovaries (Manning, pers. comm.). Generally, the females are larger than the males. Modal size range is 225-250 mm, at which size males weighed 125-250 g and females 180-330 g (Table 3).

Mantis shrimps are not commercially exploited in Plaridel or other local markets of Misamis Occidental. Rarely, they are sold at ₱45-50/kg (US$1 = ₱20). In Sitangkai Islands, mantis shrimps are commercially exploited at ₱5-10/pair or ₱10-20/kg.

Water sampling for salinity, pH, turbidity, dissolved oxygen and temperature was carried out during lowest tides in Polo Pt., Plaridel at night. In Sitangkai Islands, water sampling and temperature readings were done at daytime. All parameters showed a narrow range and did not seem to be the factors exerting a limiting effect on the movement of these animals (Table 4).

The substrate within the burrow of mantis shrimps is of fine coral-sand dominated by 40-60 μm grain size with average content of 58.51%. Preference for grain size may rest on the capacity of grains to retain water and their suitability for burrowing. Fine sand is an excellent buffer against abrupt changes of temperature, salinity and dessication (Nybakken 1982).

Mantis shrimp burrows were frequently found in seagrass areas. These grasses perhaps provide camouflage for the organism, while fish and other animals seeking shelter in the seagrass may serve as food. Four species of seagrass occur in the sampling areas: *Thalassia becarii, Halodule pinifilia, Enhalus acoroides* and *Halophila ovalis*.

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Table 1. Distribution and abundance of mantis shrimp(s) and burrows at various 1-ha sites at Polo Pt. and Sitangkai.

	Polo Pt.			Sitangkai	
Site	No. burrows	No. shrimp	Site	No. burrows	No. shrimps
1	3	3	1	13	10
2	6	7	2	9	6
3	4	6	3	12	8
4	3	3	4	8	7
5	0	0	5	7	5
6	5	6	6	1	1
7	0	0	7	0	0
8	4	4	8	2	2
9	8	10	9	3	3
10	2	3	10	0	0
11	2	2			
12	1	1			
13	3	6			
14	1	1			
15	1	1			
16	3	4			
Total	46	57	Total	55	42

Table 2. Size class frequency of burrows with captured shrimp in Polo Pt., Plaridel, Misamis Occidental.

Burrow's diameter range (mm)	No. of burrow	No. of catch	Size range of carapace length (mm)	
			Male	Female
50 – 55	5	1	41	–
56 – 61	10	7	34 – 45	34 – 36
62 – 67	4	1	–	36
68 – 73	13	11	35 – 49	42 – 48
74 – 79	4	6	43 – 45	44 – 49
80 – 85	6	7	32 – 33	43 – 48
86 – 91	1	0	–	–
92 – 97	3	5	44 – 49	41 – 50
Total	46	38		

Table 3. Size composition (total length) in relation to weight range of mantis shrimp.

Total length (mm)	Frequency	Weight range (g)	
		Male	Female
100-125	1	50	–
125-150	1	–	50
150-175	1	–	85
175-200	7	80-130	100-125
200-225	11	100-200	130-165
225-250	28	125-250	180-330
250-275	15	72-320	190-300
275-300	12	200-395	350-490
Total no. of sample size	78		

Table 4. Tabulation of the range values of the physicochemical parameters in two stations.

	Salinity (ppt)		Temperature (°C)			pH		Turbidity Reg.		D.O. (ppm)	
	Surface	Burrow mouth	Air	Water	Sediment	Surface	Burrow mouth	Surface	Burrow mouth	Surface	Burrow mouth
Polo Pt.	30.0 to 37.4	30.0 to 37.3	26.3 to 30.5	26.8 to 31.5	27.0 to 30.5	7.7 to 8.3	7.6 to 18.3	7.9 to 16.8	8.0 to 16.8	4.0 to 14.3	1.9 to 7.5
Sitangkai	32.0 to 36.0	32.0 to 36.0	29.5 to 31.8	29.0 to 31.8	30.0 to 31.0	8.2 to 8.7	8.2 to 8.3	13.0 to 18.0	11.0 to 15.3	7.3 to 11.7	7.2 to 10.2

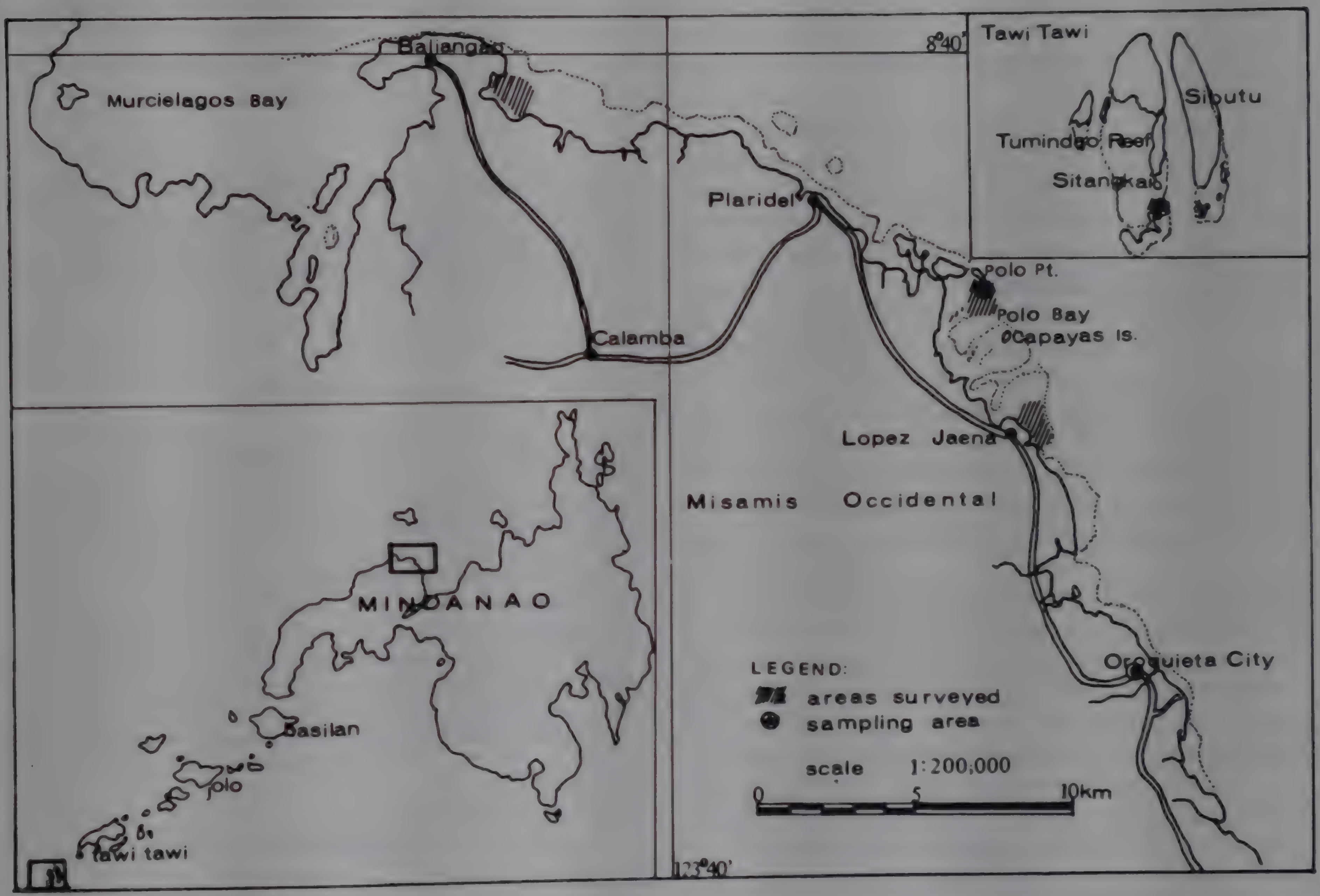
Baliangao
Murcielagos Bay
8°40'
Tawi Tawi
Sibutu
Tumindao Reef
Sitangkai
Plaridel
Polo Pt.
Polo Bay
Capayas Is.
Calamba
Lopez Jaena
Misamis Occidental
MINDANAO
Basilan
Jolo
Tawi tawi
Oroquieta City
LEGEND:
areas surveyed
sampling area
scale 1:200,000
0
5
10km
123°40'

Fig. 1. Areas surveyed in Misamis Occidental and Tawi Tawi.

The Offshore Environment of Tungkang, Southwest Taiwan. II. Macrobenthos*

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Abstract

The macrobenthic fauna and sediments off the Tungkang coast were studied from samples at 30 stations in the autumn of 1983 with an Ekman-Birge grab and Tamura's sand grab. Sediments were almost fine sand or silt-clay mud. Numerical density of individuals ranged from 26 to 3,006/m^2. Number of species in each station was 1-18 with about nine species on the average. The dominant fauna was polychaetes with 31 species of Spionidae, Magelonidae, Capitellidae, Nephtyidae and Goniadidae. Mollusca included Naticidae, Dentaliidae, Tellinidae spp. and a dominant bivalve of Verenidae, *Veremolpa scabra*. Crustacea included many amphipods belonging to Ampeliscidae and Corophiidae, with a few paranthurid Isopoda, Anomura and Brachyura. The relationship between sediments and animals is discussed in reference to the habitat of taxonomic groups and species.

Introduction

There are very few reports on the macrobenthic fauna in waters around Taiwan, although several investigators have worked on their distribution and systematics in various parts of the East China Sea and adjacent waters (Paik 1975, 1980; Wo Baoling et al. 1975; Lee 1976; Hong and Lee 1983; Lee et al. 1983). As the coastal zone of southwest Taiwan is known to be intensively fished for shrimp, miscellaneous biological information is highly expected to provide useful ecological data for fishery management. Knowledge of benthic communities contributes significantly to demersal fish production and related problems.

This paper describes the macrobenthic fauna in the Tungkang region, the typical coast of southwest Taiwan and the composition of benthic communities in relation to sedimentation and the species habitats. An environmental survey of water and pollution was carried out at the same time by other members of Tungkang Marine Laboratory.

Materials and Methods

The study area is the offshore waters in the Tungkang coast (22° 25'N, 120°25'E) located 20 km southeast from Kaohsiung City (Fig. 1). The coast is open to the East China Sea and subjected to moderate wave washing and coastal current. Kaoping Hsi, one of the largest rivers in Taiwan and two smaller rivers, Tungkang Hsi and Linpien Hsi, flow into the coastal waters. A steep bottom slope faces the mouth of Kaoping Hsi.

Thirty sampling stations were arranged approximately 2.8 km apart in a grid pattern from the northwest of the mouth of Kaoping Hsi, across it and Tungkang town, to the southeast of the mouth of Linpien Hsi. Several stations were established in the estuaries of these rivers and data from 22 of these stations were finally used in this report.

The survey was carried out on 2-3 November 1983 with the use of a chartered fishing vessel from Tungkang. Sediment samples were obtained three times at each station with an Ekman-Birge grab (0.04 m^2) or Tamura's sand grab (0.05 m^2). Samples were treated immediately upon arrival at the port. A small portion of the sediment was saved for sediment analyses. The remainder was weighed before washing the contents through a sieve of 0.5-m^2 aperture. The animals collected from the sieve were fixed in 10% formalin. After sorting, the specimens were preserved in 5% neutralized formalin, identified, counted and weighed, the total of each station recorded in wet weight.

Species diversity of each station was calculated by Shannon-Weaver's index as follows:

$$H' = -\sum_{i=1}^{s} Pi \log_2 Pi$$

where H^1 is the index of species diversity, S is the number of species, and Pi is the proportion of total sample belonging to *i*th species.

In grain size analysis of sediment, fractions from coarse sand to fine sand were measured by sieve method, and silt-clay fraction was estimated from weight loss through a 0.063-m^2 sieve. Median grain diameter was expressed as Phi scale. Organic matter of sediment was measured by the ignition method at 600°C for two hours.

Results and Discussion

The numerical densities at 22 sampling stations were 26-3,006 individuals/m^2, the average being 386.6 individuals/m^2 showing a low production in shallow waters facing the open sea. The high densities of Foraminifera at Station 13 and Station 27 were not considered because it was doubtful if these organisms are of the benthic assemblage. The biomass also showed comparatively low figures of 0.1-7.0 g/m^2 in wet weight with an average of 1.8 g/m^2 . From each station, 1 to 18 species were obtained with 8.7 species as the average. There were 67 species in all, the major group being Polychaeta with 31 species. Values of specific diversity were 0-3.52 with a low average of 2.46 which is a characteristic feature of offshore waters.

The major numerical components were Polychaeta (61.8%), Mollusca (15.3%) and Crustacea (17.1%) which comprised 94.2% of the total. Polychaeta included species belonging to Spionidae, Magelonidae, Capitellidae, Nephtyidae, Goniadidae and others, including a few opportunistic species such as *Capitella capitata*. Mollusca included Naticidae, Dentaliidae, Tellinidae and a dominant species of Veneridae, *Veremolpa scabra*. Crustacea included many amphipod species of Ampeliscidae and Corophiidae, with a few paranthurid Isopoda, Anomura and Brachyura. Table 1 shows that nine species were identical with previously recorded species from other districts of the East China Sea and neighboring waters; namely, *Nephthys ciliata, Prionospio japonicus, Cirriformia tentaculata, Capitella capitata, Sternaspis scutata, Macoma tokyoensis, Theora lubrica (= Th. fragilis)* and *Photis longicaudata* in Korea (Paik 1975, 1980; Lee 1976; Hong and Lee 1983; Lee et al. 1983) and *Glycera lancadivae* in Xisha Islands of the South China Sea (Wu Baoling et al. 1975).

The relation of benthonic habitat to environment through the presence of trophic species was presumed from the literature about the same genus or other taxonomic groups (Hunt 1925; Enequist 1950; Griffiths 1976; Fauchald and Jumars 1979; Morton and Morton 1983), in accordance with the division of Dorsey (1982). Table 1 shows a great variety of carnivores and omnivores which accounted for 35.8% of the total species. Deposit feeders amounted to 29.9%, deposit-suspension feeders, such as tubiculous species especially in Polychaeta made up 17.9%, suspension feeders 10.4%, and unknown 6.0%. The percentage of deposit-suspension feeders is low compared with 82.7% obtained from the polluted area near the sewage-treatment farm recorded by Dorsey (1982). The Tungkang area can therefore be considered to have unpolluted offshore waters.

Sediments had a median grain diameter of 2.06-6.64 by ϕ-scale, that is, almost fine sand and very fine sand, with a number of silt-clay materials. Values of ignition loss as an indicator of organic matter ranged from 1.15 to 5.38%. Organic matter plotted against median grain diameter of the sediment on a semi-log scale, has usually a straight line relationship. Most organic matter is associated with small grains; their larger surface area permits more adsorption of microorganisms. Data from our survey also show the same relationship (Fig. 2), but the tangent of the straight line was lower than an example from polluted coastal waters (Kuwabara 1985).

The distribution of median grain diameter (Md) is shown with the numerical density of animals in Fig. 3. High densities of animals have been found northwest of Kaoping Hsi and in the estuary of Linpien Hsi. The former is dominated by a species of suspension feeder, *Veremolpa scabra*, and a few deposit feeders. In the latter river, the mouth area was mainly occupied by an amphipod, *Kamaka* sp., and an unknown Gastropoda; on the other hand, the estuary was dominated by a spionid polychaete, *Prionospio japonicus*, which was recorded from the inner area of a bay in Korea (Lee 1976) and the upper reaches of two large rivers in Japan (Tsuda and Kitagawa 1965; Kuwabara and Akimoto 1985).

Bottom depth showed a fine relationship with median grain diameter and with specific diversity index (H') shown in Fig. 4. These two induced factors are considered to be correlated. The major animal groups were concentrated in a few ranges of grain size (Fig. 5), especially at Md (ϕ) 3.7-4.6. The pattern was clear for the major taxonomic divisions of Polychaeta, Errantia and Sedentaria (Fig. 6).

The results were applied in the ranking of stations according to environmental conditions and biological characteristics (Table 2).

Group I is composed of two estuarine stations, Stations 25' and 27, where the sediments are coarser sand and communities are characterized by fewer species or low specific diversity. The majority of species are Polychaeta, of which *Prionospio japonicus*, an inhabitant of brackishwater, is dominant and an opportunistic species, *Capitella capitata*, is secondarily dominant.

Group II is typical of offshore waters in the survey; the majority of stations are included in this group. It has medium depth, very fine sand and medium ignition loss. Biological characters of many items are near the average. A bivalve, *Veremolpa scabra*, is the most dominant species, in spite of its low numerical abundance at 26.5%. An amphipod suspension feeder, *Ampelisca brevicornis*, an opportunistic deposit feeder, *C. capitata*, and a few deposit-suspension feeders follow the dominant species, with a predator, *Nephtys ciliata*. The dominance of suspension feeders means a remarkable utilization of organic particles supplied by river inflow. Dominant coexistence of *Ampelisca brevicornis* and the other species of *Veremolpa* has also been recorded from the sand bottom off the mouth of major rivers in Japan (Hayashi 1983).

Group III covers the deepest zone in the survey area with silt-clay which had higher ignition loss. It is characterized by low numerical density and lower specific diversity. Inhabitants of this muddy bottom are a carnivore, *Nephtys dibranchis*, as the most dominant species and several deposit feeders, *Megelona cincta*, *Lumbrineris meteorana*, *Cirriformia tentaculata* and others.

Regional separation of closely-related species was observed between *Nephtys ciliata* in Group II and *N. dibranchis* in Group III, as in the distribution of four species of *Nephtys* in the estuary of the Netherlands (Wolff 1971). Group IV is an exceptional estuarine station, where only one species, *Golfingia* sp., a sipunculid, was found in muddy bottom with the highest ignition loss.

The characteristics of macrobenthic communities in the offshore waters of Tungkang area are generally recognized from the perspective of Group II (Table 2). From the values of each item, it is suggested that organic matter derived from the precipitation of river-borne substances has much influence on the ecosystem of these benthic communities.

The foods of penaeid shrimp are the major taxonomic groups of macrobenthos (Ikematsu 1963). Epifaunal suspension feeder and deposit-suspension feeders, as in Group II, are more preferable than infaunal deposit feeders to shrimp.

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*Tungkang Marine Laboratory Contribution A No. 67.

Table 1. Species of benthic macrofauna collected at Tungkang region and their trophic type.

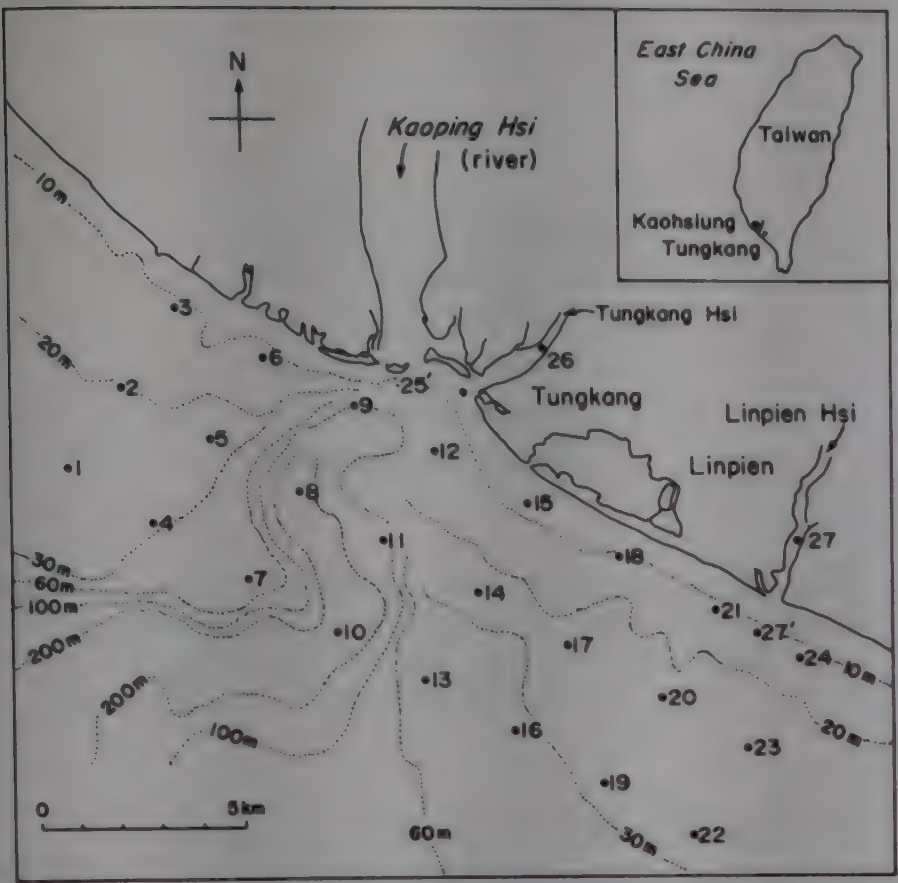
Species	Trophic type	Species	Trophic type
* Foraminifera	D/S?	Mollusca	
Sipunculida		*Mammilla mammata*	C
Golfingia sp.	O	*Siphonalia* sp.	C or O
Polychaeta		*Turricula javana*	C
Aphroditidae sp.	C	*Cadulus (Platyschides) opportumus*	D
Sigalion mathildae	C	• Dentaliidae sp.	D
Eulepethus hamifera	C	• *Veremolpa scabra*	S
Pisione sp.	D	*Theora lubrica*	D
Phyllodoce gröenlandica	C	*Nitidotellina minuta*	D/S
Sigambra sp.	C	*Macoma contabulata*	D/S
Neanthes sp.	O	*Macoma tokyoensis*	D/S
Nephtys (Nephtys) ciliata	C	*Cuspidaria nobilis*	C
Nephtys (Aglaophamus) dibranchis	C	• unknown Gastropoda	?
Paralacydonia paradoxa	O	Crustacea	
Glycinde armigera	C	Mysidae sp.	D/S
Goniada emerita	C	*Apseudes* sp.	D ?
Hemipodus sp.	C	*Cyathura indica*	D
Glycera lancadivae ?	C	*Gnathia* sp.	?
Lumbrineris meteorana	D	*Ampelisca cyclops*	S
Lumbrineriopsis sp.	D	*Ampelisca brevicornis*	S
Onuphis eremita	C	*Phoxocephalus tenuipes* ?	O
Eunice sp.	O	*Urothoe grimaldi*	D
Pseudopolydora kempi	D/S	*Harpiniopsis* sp.	D
• *Prionospio japonicus*	D/S	*Idunella curvidactyla*	D/S
• *Paraprionospio* sp.	D/S	*Maera hirondellei* ?	D
Magelona cincta	D/S	*Photis longicaudata*	D
Cirriformia tentaculata	D/S	*Ericthonius pugnax*	S
Orbinia sp.	D	*Siphonoecetes kroyeranus* ?	S
Scoloplos (Leodamas) johnstonei	D	• *Kamaka* sp.	S ?
Aricidea suecica	D/S	*Callianassa japonica*	D
Armandia leptocirrus	D	*Laomedia astacina*	?
• *Capitella capitata*	D	*Alpheus* sp.	O ?
Sternaspis scutata	D	*Albunea symnista*	O ?
Maldanidae sp.	D	*Tritodynamia* sp.	O ?
Potamilla sp.	S	Ohiuroidea	
		Amphioplus aucistrotus	D
		Osteichthyes	
		Bregmaceros japonicus	?

C = carnivore, O = omnivore, S = suspension feeder, D = deposit feeder, D/S = deposit-suspension feeder, ? = unknown.

Asterisk indicates a numerical density larger than 100 individuals/m^2 at one or more stations.

Table 2. Sediment conditions, biological characteristics and dominant species ranked by the abundance of each grain-size group.

Group		I	II	III	IV
No. of station		2	13	6	1
Depth range	(m)	2-3	11-31	21-34	4
Md (ϕ) of sediment	(mean) (range)	2.40 2.06-2.74	4.11 3.72-4.51	6.64 <	6.64 <
Ignition loss (%)	(mean) (range)	2.93 2.28-3.57	3.20 1.15-3.88	4.00 3.35-5.06	5.38
Nos. of species	(mean) (range)	6.0 2-8	10.6 5-18	7.2 3-10	1
Numerical density (no./m^2)	(mean) (range)	1,522.5 39-3,006	347.6 117-1,102	182.7 104-286	26
Specific diversity (H')	(mean) (range)	1.21 0.92-1.50	2.81 2.03-3.52	2.52 1.50-3.18	0
Biomass (g/m^2)	(mean) (range)	2.3 0.1-4.4	2.1 0.4-7.0	1.0 0.3-2.3	1.8
Numerical composition of animal group (%)	Polychaeta Mollusca Crustacea Miscellaneous	93.1 2.6 4.3 0	55.4 23.2 20.0 1.4	75.3 4.9 17.9 1.9	0 0 0 100
Numerical composition of dominant species (%)		*Prionospio japonicus* 69.6% *Capitella capitata* 11.9% *Kamaka* sp. 8.5% unknown Gastropoda 4.3% *Paraprionospio* sp. 2.6%	*Veremolpa scabra* 26.5% *Paraprionospio* sp. 17.7% *Capitella capitata* 7.0% *Ampelisca brevicornis* 4.4% *Magelona cincta* 3.6% *Nephtys ciliata* 3.0% *Cyathura indica* 3.0%	*Nephtys dibranchis* 10.7% *Magelona cincta* 8.3% *Lumbrineris meteorana* 7.1% *Cirriformia tentaculata* 7.1% *Capitella capitata* 7.1% *Scoloplos johnstonei* 6.9% *Paraprionospio* sp. 6.3%	*Golfingia* sp. 100%


N
Kaoping Hsi
(river)
East China Sea
Taiwan
Kaohsiung
Tungkang
Tungkang Hsi
Tungkang
Linpien Hsi
Linpien
10m
20m
30m
60m
100m
200m
0
5km
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
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24
25
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27
27'

Fig. 1. The sampling sites, southwest Taiwan.

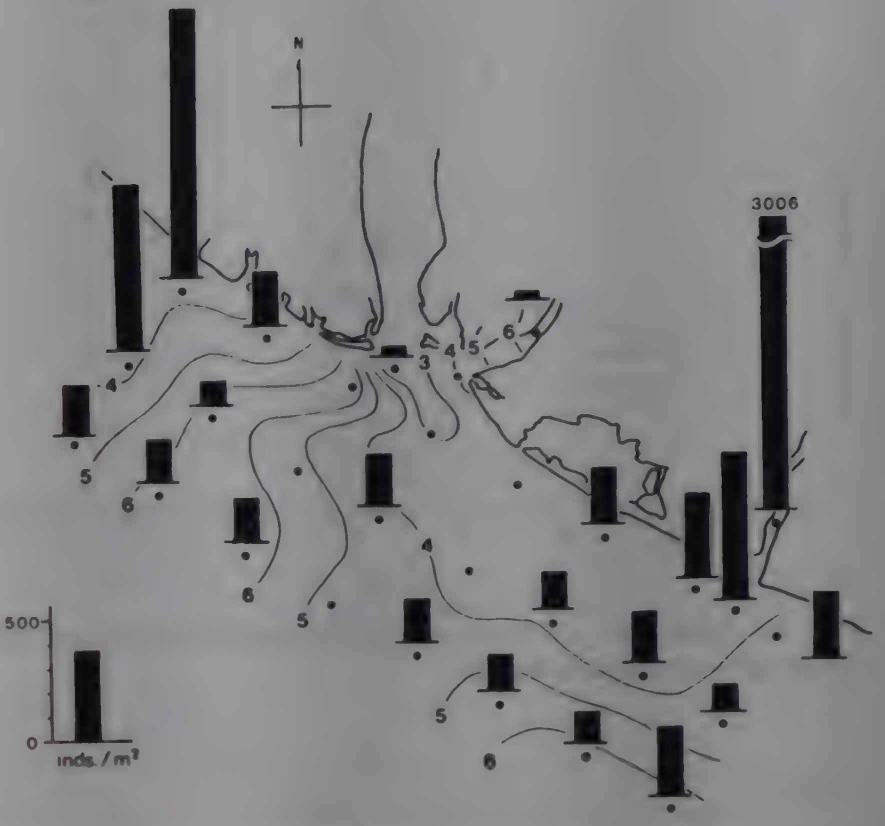
N
3006
500
0
inds./m²
3
4
5
6

Fig. 3. Distribution of numerical density (no./m^2) with contours of Md (ϕ).

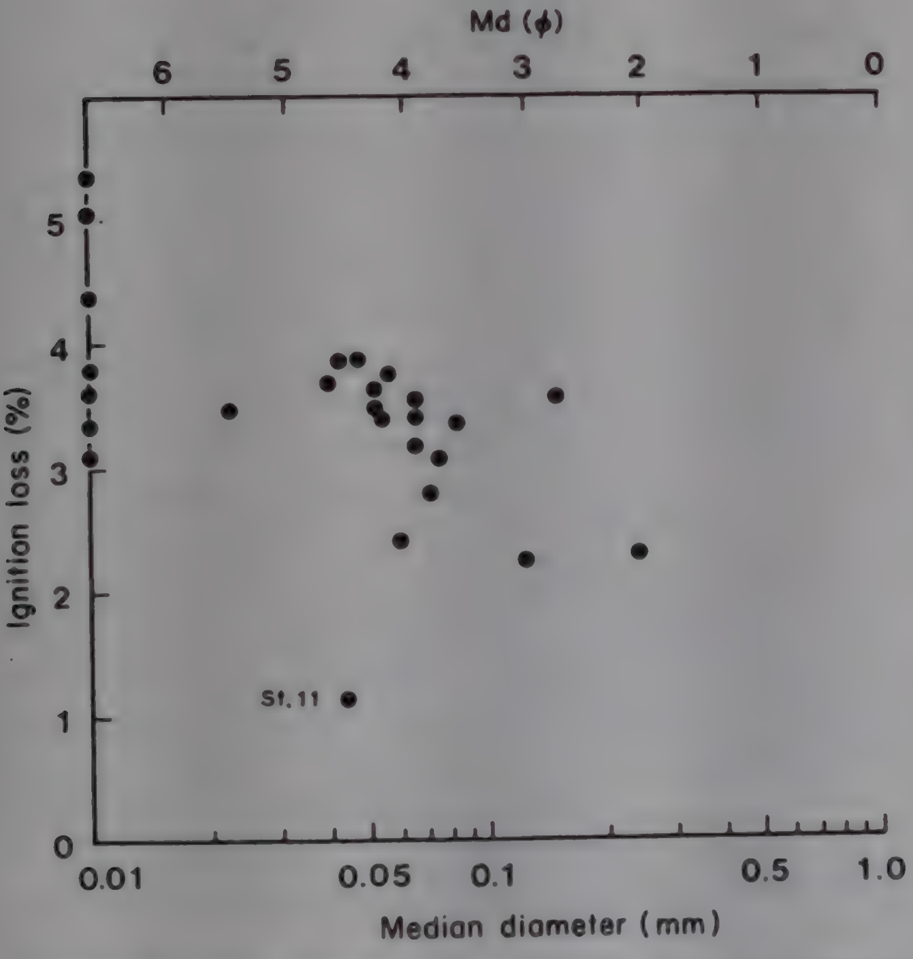

Fig. 2. Correlation between median grain diameter and ignition loss in sediments (Station 11: exceptional station near the steep bottom slope).

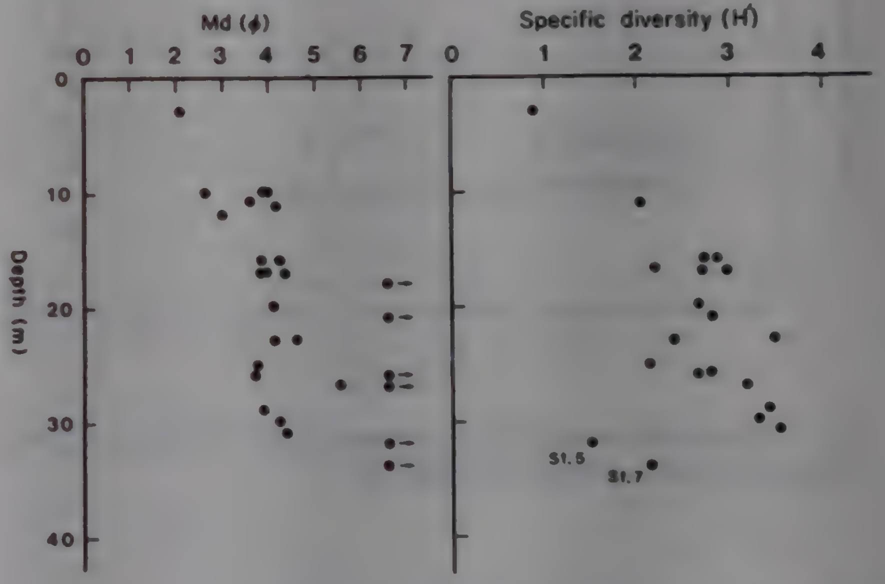

Fig. 4. Changes in Md (ϕ) and specific diversity (H$'$) corresponding to the increase of bottom depth. (Arrow: over to 6.64. Stations 5 and 7: exceptional stations near the steep bottom slope).

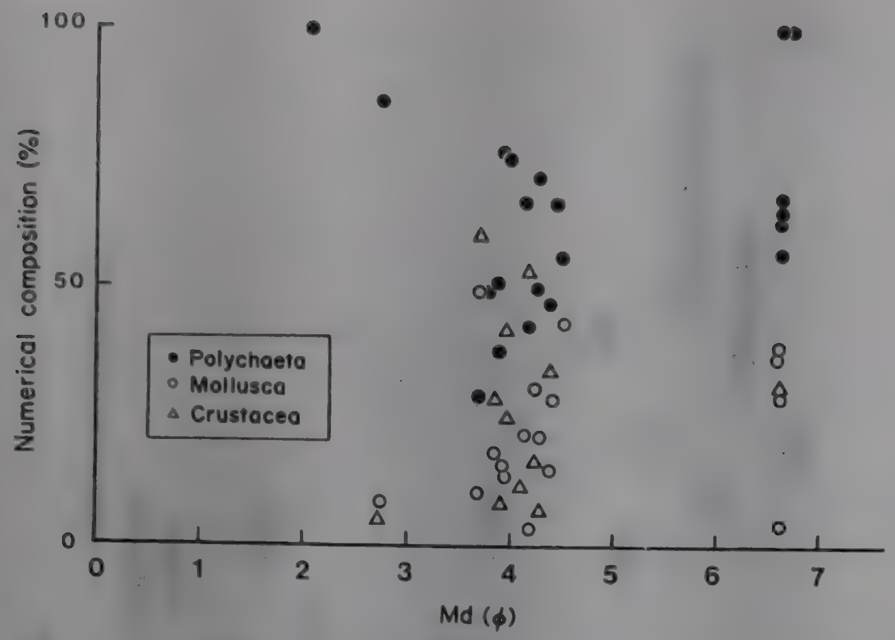

Fig. 5. Numerical composition of Polychaeta, Mollusca and Crustacea in relation to Md (ϕ).

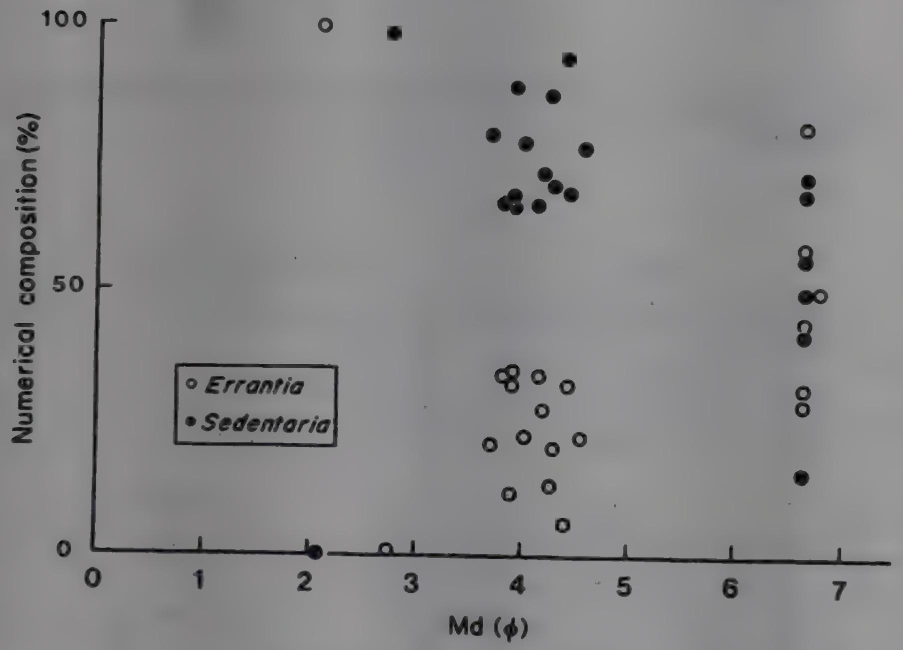

Fig. 6. Numerical composition of Polychaeta Errantia and Polychaeta Sedentaria in relation to Md (ϕ).

Elasmobranch Fishes in the Nagasaki Fishmarket

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The west sea area of Kyushu including the extensive continental shelf and slope of East China Sea is favored by the many islands and complicated coastlines and a gigantic convergence between the Tsushima warm current and a water mass of the southern Yellow Sea. In consequence, many kinds and large numbers of fauna and flora inhabit the area. A number of different fishing techniques have operated in this sea area, including trawl and bull trawl, drift and set longlines, purse seines, gill nets, stationary nets, pole and line and whaling. These fishing grounds encompass the coastal waters of west Kyushu, Yellow Sea, East China Sea, northern South China Sea and southwestern Pacific. The center of these fisheries since the last century has been Nagasaki fishery harbor. More kinds of fishes have been collected in the Nagasaki fishmarket than in any other place in Japan.

Siebold (1842) and Glover (1912-1933) had published "Fauna Japonica" and "Fishes of Southern and Western Japan", respectively, which included many fish species landed at Nagasaki. Makihata (1967), Matsuo (1968), Okano (1982) and others, as well as the Faculty of Fisheries, Nagasaki University, have investigated the sharks and rays landed in that fishmarket.

The following list contains the results of investigations on 61 species of rays, including 13 genera and 8 families.

Recently, the authors used set longlines for deep-sea sharks in the adjacent waters of Nagasaki and collected five species of sharks, and added three species of three genera, *Squaliolus laticaudatus*, *Cirrhigaleus barbifer* and *Deania eglantia*, to the list. *Isistius brasiliensis* was judged to be a member of the family Squalidae instead of family Dalatiidae.

- Hexanchidae
    - *Hexanchus*
        - *H. griseus* (Bonnaterre)
    - *Heptranchias*
        - *H. perlo* (Bonnaterre)
    - *Notorhynchus*
        - *N. cepedianus* (Péron)
- Heterodontidae
    - *Heterodontus*
        - *H. japonicus* (Duméril)
        - *H. zebra* (Gray)
- Scyliorhinidae
    - *Galeus*
        - *G. eastmani* (Jordan et Snyder)
        - *G. sauteri* (Jordan et Richardson)
    - *Cephaloscyllium*
        - *C. umbratile* Jordan et Fowler
    - *Scyliorhinus*
        - *S. torazame* (Tanaka)
    - *Halaelurus*
        - *H. buergeri* (Müller et Henle)
- Orectolobidae
    - *Orectolobus*
        - *O. japonicus* Regan
    - *Cirrhoscyllium*
        - *C. expolitum* (Smith et Radcliffe)
    - *Stegostoma*
        - *S. fasciatum* (Hermann)
    - *Chiloscyllium*
        - *C. colax* (Meuschen)
- Sphyrnidae
    - *Sphyrna*
        - *S. zygaena* (Linnaeus)
        - *S. lewini* (Griffith et Smith)
- Lamnidae
    - *Carcharodon*
        - *C. carcharias* (Linnaeus)
    - *Isurus*
        - *I. oxyrinchus* Rafinesque
- Cetorhinidae
    - *Cetorhinus*
        - *C. maximus* (Gunnerus)
- Alopiidae
    - *Alopias*
        - *A. pelagicus* Nakamura
        - *A. superciliosus* (Lowe)
- Odontaspidae
    - *Odontaspis*
        - *O. taurus* (Rafinesque)

- Triakidae
  - *Triakis*
    - *T. scyllia* Müller et Henle
  - *Mustelus*
    - *M. manazo* Bleeker
    - *M. griseus* Pietschmann
  - *Hemitriakis*
    - *H. japonica* (Müller et Henle)
    - *H. hyugaensis* (Miyosi)
  - *Proscyllium*
    - *P. habereri* (Hilgendorf)
- Carcharhinidae
  - *Carcharhinus*
    - *C. obscurus* (Lesueur)
    - *C. longimanus* Poey
    - *C. plumbeus* (Nardo)
    - *C. dussumieri* (Valenciennes)
    - *C. limbatus* (Valenciennes)
    - *C. falciformis* (Bibron)
    - *C. melanopterus* (Quoy et Gaimard)
    - *C. leucus* (Valenciennes)
    - *C. sorrah* (Valenciennes)
    - *C. albimarginatus* (Rüppell)
    - *C. brevipinna* (Müller et Henle)
  - *Prionace*
    - *P. glauca* (Linnaeus)
  - *Galeocerdo*
    - *G. cuvier* (Pěron et Lesueur)
  - *Hypoprion*
    - *H. macloti* (Müller et Henle)
  - *Rhizoprionodon*
    - *R. acutus* (Rüppell)
  - *Scoliodon*
    - *S. laticaudus* (Müller et Henle)
  - *Hemigaleus*
    - *H. macrostoma* Bleeker
- Pristiophoridae
  - *Pristiophorus*
    - *P. japonicus* Gunther
- Squatinidae
  - *Squatina*
    - *S. japonica* Bleeker
    - *S. nebulosa* Regan
- Squalidae
  - *Squalus*
    - *S. mitsukurii* Jordan et Fowler
    - *S. japonicus* Ishikawa
    - *S. brevirostris* Tanaka
    - *S. blainvillei* (Risso)
  - *Centrophorus*
    - *C. atromarginatus* Garman
  - *Isistius*
    - *I. brasiliensis* (Quoy et Gaimard)
  - *Etmopterus*
    - *E. frontimaculatus* Pietschmann
    - *E. lucifer* Jordan et Snyder
    - *E. molleri* Whitely
    - *E. brachyurus* (Smith et Radcliffe)
  - *Squaliolus*
    - *S. laticaudatus* Smith et Radcliffe
  - *Cirrhigaleus*
    - *C. barbifer* Tanaka
  - *Deania*
    - *D. eglantina* Jordan et Snyder

Rajiformes

- Dasyatidae
  - *Dasyatis*
    - *D. akajei* (Müller et Henle)
    - *D. bennetti* (Müller et Henle)
    - *D. melanospilos* (Bleeker)
    - *D. ushiei* Jordan et Hubbs
    - *D. zugei* (Müller et Henle)
    - *D. uarnak* (Forsskål)
  - *Gymnura*
    - *G. japonica* (Temminck et Schlegel)
    - *G. poecilura* (Shaw)
  - *Urolophus*
    - *U. aurantiacus* Müller et Henle
- Rajidae
  - *Raja*
    - *R. kenojei* Müller et Henle
    - *R. hollandi* Jordan et Richardson
    - *R. tengu* Jordan et Fowler
    - *R. porosa* Günther
    - *R. acutispina* Ishiyama
    - *R. macrophthalma* Ishiyama
- Pristidae
  - *Pristis*
    - *P. cuspidatus* Latham
- Torpedinidae
  - *Narke*
    - *N. japonica* (Temminck et Schlegel)
- Mobulidae
  - *Mobula*
    - *M. japonica* (Müller et Henle)
- Platyrhinidae
  - *Platyrhina*
    - *P. sinensis* (Bloch et Schneider)
- Myliobatidae
  - *Myliobatis*
    - *M. tobijei* Bleeker
  - *Aetobatus*
    - *A. narinari* (Euphrasen)

- Rhinobatidae
  - *Rhinobatos*
    - *R. schlegelii* Müller et Henle
    - *R. hynnicephalus* Richardson
  - *Rhynchobatus*
    - *R. djiddensis* (Forsskål)
  - *Rhina*
    - *R. ancylostoma* Bloch et Schneider

Indices of Overall Growth Performance of 100 Tilapia (Cichlidae) Populations*

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Abstract

Four indices for comparing the overall growth performance of fishes (ω, P, ϕ, and ϕ') proposed by various authors are assessed, based on growth parameter estimates in 100 populations of tilapia in fifteen species of the genera *Tilapia*, *Sarotherodon* and *Oreochromis*, from inland waters in Africa and Asia. The best index, i.e., the one whose distribution was most similar to a normal distribution was ϕ' ($= \log_{10}K + 2\log_{10}L_\infty$); the worst index was ω ($K = L_\infty$). The best growth performance in all populations investigated was in *Oreochromis niloticus* from Lake Kainji, Nigeria, the worst in *O. mossambicus* from Lake Sibaya (South Africa). Some theoretical and practical implications of these findings are discussed.

Introduction

Growth comparisons of fish based on a single parameter have been found to be misleading (Pauly 1979; Kimura 1980; De Merona 1983; Moreau et al. 1985). Several authors have proposed indices of overall growth performance based on two parameters (Gallucci and Quinn 1979; Pauly 1979; Munro and Pauly 1983; Pauly and Munro 1984). These indices all take account of the feature that "the growth curves of different fishes cannot be compared directly because the curves themselves are produced by growth rates that are constantly changing with time and size" (Pauly 1979). Hence, these indices all relate to a given part of a growth curve, selected as representative of overall growth performance.

This contribution compares the growth performance of 100 "populations" of tilapias (males and females are treated here as separate "populations" whenever their growth parameters had been, or could be estimated separately). Altogether fifteen species, belonging to the genera *Tilapia*, *Sarotherodon* and *Oreochromis* (Cichlidae: Teleostei) are covered, most of them from African inland waters. The aims are:

- to review briefly and explore the characteristics of some indices of growth performance that have been proposed in the literature; and
- to identify those tilapia populations which, in nature at least, have the highest growth performance, and hence to help identify African strains of tilapia with aquaculture potential in Asia.

Throughout this paper, the von Bertalanffy Growth Formula (VBGF) is used to express the growth of fish (von Bertalanffy 1957); it has, for growth in length the form

$$L_t = L_\infty(1-\exp(-K(t-t_0)) \quad \text{... 1)}$$

and for growth in weight

$$W_t = W_\infty(1-\exp(-K(t-t_0)))^3 \quad \text{... 2)}$$

where L_t (or W_t) are length (or weight) at age t, L_∞ (or W_∞) the asymptotic size, K and t_0 are constants with dimensions l/time and time, respectively. Four indices of growth performance are presented and evaluated here.

Parameter ω. Gallucci and Quinn (1979) pointed out the need for independence between L_∞ and K to (1) improve the quality of estimates; (2) increase the flexibility of the VBGF; and (3) allow for statistical comparison of growth performance. They proposed an alternative to equation (1), i.e.,

$$L_t = (\omega/K)\,(1-\exp(-K(t-t_0)) \quad \text{... 3)}$$

where

$$\omega = K.\,L_\infty \quad \text{... 4)}$$

Gallucci and Quinn (1979) suggested that the parameter ω, which expresses growth rate (dl/dt) at t_0, is suited for comparison, mainly because its distribution is more normal than that of K or L_∞ taken separately. The ω index has been used by Kipling (1983), Appeldoorn (1983) and Beukema and Meehan(1985).

Parameter P. Pauly (1979) formulated the following criteria for an index of growth performance of fish: (1) it should relate to growth in weight, so as to allow comparison of species with different shapes; (2) it should consist of a single value and be easy to compute; and (3) it should be biologically interpretable.

The first derivative of equation (2), which expresses the growth rate of fish, has a single maximum $(dw/dt)_{max}$ whether it is plotted against time or against weight. Therefore, the growth rate at the inflexion point (W_i) of equation (2) can be used as standard for comparisons within and between species of different shapes. Note also that the fish of weight W_i usually represent, in nature, the bulk of their cohort (Philippart 1977). Compare with ω which expresses dl/dt at length L = 0 and hence at a cohort weight of zero.

Growth rate at the inflexion point of equation (2) is given by

$$dw/dt_{max} = (4/9)\ KW_\infty \qquad \text{... 5)}$$

Hence one can define

$$P = \log_{10} (K.W_\infty) \qquad \text{... 6)}$$

whose antilog is directly proportional to $(dW/dt)_{max}$. Because of this, P is the only one of the four growth indices presented here whose value can be computed even when growth parameters are not available. In such cases, P can be computed from the slope of the steepest part of a weight growth curve.

Parameter ϕ. The parameter ϕ, introduced by Munro and Pauly (1983) is based on the growth parameter estimates compiled and analyzed by Pauly (1978, 1979,; Table 1). It is defined by

$$\phi = \log_{10}K + (2/3) \log_{10} W_\infty \qquad \text{... 7)}$$

Parameter ϕ'. Pauly (1979), working with a large compilation of growth parameter estimates (Pauly 1978) noted that the relationship between K and L_∞ is, between different fish stocks of the same species, not one of strict proportionality (as assumed, e.g., by Gallucci and Quinn 1979). Rather, this relationship is, on the average

$$\text{Log}_{10}K = \phi' - (2/3)\log_{10}L_\infty{}^3 \qquad \text{... 8)}$$

which leads to the definition

$$\phi' = \log_{10}K + 2 \log_{10}L_\infty \qquad \text{... 9)}$$

Table 1 gives a summary of the data which lead to the mean slope estimate of 2/3 for equation (7). The parameter ϕ' has been used, among others, by Pauly (1980), Munro and Pauly (1983) and Pauly and Munro (1984), who also introduced the new symbol ϕ' to replace "a" used earlier.

The relationship of ϕ' to ϕ is given in Table 2 with the interrelationships and dimensions of all four growth performance indices presented here.

Materials and Methods

Table 3 presents the growth parameters used to test the four indices of growth performance. They were either taken from the reference cited, or computed, using standard methods, from size-at-age data in the cited literature. When necessary, length-to-weight conversions were performed.

For each index, the arithmetic mean, standard deviation and coefficient of variation, in percentages (C.V. = s.d. x 100/mean), were computed for important species in Table 3 (Table 4) and for the whole data set (Fig. 1).

The properties expected from the "best" index of overall growth performance are:

- it should be normally distributed when applied to a large number of populations belonging to closely-related taxa (such as the tilapias); and
- its variance should decrease as one descends from higher to lower taxonomic levels.

Results and Discussion

As shown in Fig. 1 the distribution of P, ϕ and ϕ' values are essentially normal and rather sharply peaked suggesting that these three indices, especially ϕ' can indeed be used as indices of growth performance. Table 4 gives results for six species. Parameter ϕ' has the lowest C.V. values, followed by P and ϕ; ω has C.V. values 2-3 times higher than the other three indices.

Thus, our conclusion is that ϕ' has properties which make it useful as an index of overall growth performance in fish, while ω performed so badly that it should not be used for such purpose, notwithstanding the suggestion of Gulland (1983) that ω "might be useful in distinguishing differences in the early growth rate of different populations."

Note that ϕ' fulfilled both criteria for a "best" index of growth performance, as, besides having a low variance for the combined data set in Table 3, it also had a lower variance when applied to separate species (Table 4).

That ϕ' performed better than the indices based on weight (P, ϕ) is somewhat surprising, but can probably be explained by the fact that tilapia species have similar shapes, and that in most populations in Table 3, W_∞ was estimated from L_∞ using length-weight relationships not estimated jointly with the specific values of L_∞, thus adding variance to the W_∞ estimates.

Finally, the fact that ϕ' has a dimension such that fish growth performance is related to surface area (Table 2), agrees with the suggestion of Pauly (1979, 1981, 1984) that respiratory surface area (i.e., gills) and hence oxygen supply are factors limiting fish growth. However, the limiting surfaces for growth (i.e., the gills) need not grow in proportion to length^2 or weight$^{2/3}$. Rather the index ϕ' and ϕ imply length^2 or weight$^{2/3}$ only because the von Bertalanffy equation is structured around this assumption (see von Bertalanffy 1957). Thus, one would certainly obtain an equally good index of growth performance based on a power between 2/3 and 1 as occur in most fishes (Pauly 1979, 1981).

Based on the index ϕ' one can infer that *Oreochromis niloticus* and *S. galilaeus* in Lake Kainji, Nigeria, are the best-growing fishes of the lot considered here, while the worst is *O. mossambicus* in Lake Sibaya, South Africa. The irony of this is that it is now very difficult to prevent wild *O. mossambicus* from reducing, through hybridization, the growth performance of introduced strains of *O. niloticus* and *S. galilaeus* (Pullin 1983; Tanaguchi et al. 1985). Clearly, growth comparisons of wild fish stocks, based on a suitable index of overall growth performance should be performed as part of the process leading to the selection of species for transfers and introductions.

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Table 1. Statistics of the 138 empirical regressions used to derive mean slope of – 2/3 for plots of $\log_{10}K$ on $\log_{10}W_\infty$ or $\log L_\infty^3$.[a]

Type of summary data	Number of regressions	Regression statistics Mean slope[b]	s.d. of slopes	s.e. of mean slope	95% confidence interval
$\log_{10}K$ on $\log_{10}L_\infty^3$	119[b]	–0.632	0.386	0.035	–0.563 –0.701
$\log_{10}K$ on $\log_{10}W_\infty$	19	–0.714	0.279	0.064	–0.580 –0.848
Overall mean (unweighted)	138	$\boxed{-0.673}$[c]	–	–	———
Overall mean (weighted by n)	138	–0.643	$\boxed{0.374}$[d]	0.032	$\boxed{-0.581\ -0.705}$[e]

[a] Adapted from Tables X, XI and XIII a to h in Pauly (1979), based on growth parameters compiled in Pauly (1978).

[b] Including one plot each for the tilapia species *Oreochromis esculentus*, *O. niloticus* and *O. mossambicus* and excluding 7 plots with positive values of b.

[c] The plots of $\log_{10}K$ on $\log_{10}W_\infty$, although less numerous, involved growth parameter values previously checked for consistency; their mean value of b = 0.714 may thus be given the same weight as the mean b derived from the plots of $\log_{10}K$ on $\log_{10}L_\infty$.

[d] $s.d._{1+2} = \sqrt{((s.d._1{}^2(n_1-1)) + (s.d._2{}^2(n_2-1)))\,/\,(n_1+n_2-2)}$

[e] Note that 95% confidence interval includes – 2/3, i.e., the value that would be expected given the assumption of – 2/3 built in the von Bertalanffy equation (see text).

Table 2. Algebraic relationships between and dimensions of the four growth performance indices ω, P, φ' and φ.[a]

Parameter	ω	P	φ	φ'	
ω	$=\omega$	$=10^P/(a\cdot L_\infty^2)$	$=\phi-\log_{10}L_\infty-\frac{2}{3}\log_{10}{}^{a}$	$=10^{\phi'}/L_\infty$	growth rate in length (l/t)
P	$=\log_{10}(\omega\cdot a\ L_\infty^2)$	$=P$	$=\phi+\frac{1}{3}\log_{10}W_\infty$	$=\phi'+\frac{1}{3}\log_{10}W_\infty+\frac{2}{3}\log_{10}{}^{a}$	growth rate in weight $(\log_{10}(l^3/t))$
φ'	$=\log_{10}(\omega\ L_\infty)$	$=P-\frac{1}{3}\log_{10}W_\infty-\frac{2}{3}\log_{10}{}^{a}$	$=\phi-\frac{2}{3}\log_{10}{}^{a}$	$=\phi'$	growth rate in units of surface area $(\log_{10}(l^2/t))$
φ	$=\log_{10}(\omega\cdot L_\infty)+\frac{2}{3}\log^{a}$	$=P-\frac{1}{3}\log_{10}W_\infty$	$=\phi$	$=\phi'+\frac{2}{3}\log_{10}{}^{a}$	growth rate in units of surface area $(\log_{10}(l^2/t))$

[a] The parameter "a" refers to a length-weight relationship of the form $W = a\cdot L^b$ in which, for simplicity's sake, isometry is assumed (i.e., in which the value of b is set equal to 3).

Table 3. Growth parameters W_∞ (g), L_∞ (cm, standard length), K (1/yr) and indices of growth performance for 100 tilapia populations of Africa and West Asia (♂ = male; ♀ = female; absence of a symbol implies that the sexes were not separated).

Species	W_∞	L_∞	K	ω	P	ϕ	ϕ'	Area	Reference
O. aureus ♂	604	26.5	0.577	15.29	2.54	1.62	2.61	L. Kinneret	Ben Tuvia (1960)
" ♀	645	27.1	0.479	12.98	2.49	1.55	2.55	"	"
" ♂	176	16.4	0.758	12.43	2.13	1.38	2.31	L. Manzala	Payne & Collinson (1983)
"	116	14.2	1.616	22.95	2.27	1.58	2.51	L. Mariout	"
O. macrochir	1,262	31.5	0.312	9.83	2.60	1.56	2.49	Barotse Flats	Duerre (1969)
" ♂	600	25.1	0.450	11.30	2.43	1.51	2.45	Kafue Flats	Dudley (1974)
" ♀	465	22.5	0.488	10.98	2.35	1.46	2.39	"	"
"	1,628	34.3	0.266	9.12	2.64	1.57	2.50	L. Liambezi	De Merona (1983)
"	534	23.4	0.374	8.75	2.30	1.39	2.31	Kafue River	Balon & Coche (1974)
"	602	24.2	0.425	10.29	2.41	1.48	2.40	L. Itasy	Moreau (1979)
" ♂	529	23.8	0.433	10.31	2.36	1.45	2.39	L. Alaotra	"
" ♀	310	20.0	0.546	10.92	2.23	1.40	2.34	"	"
O. mossambicus ♂	408	21.6	0.358	7.73	2.16	1.29	2.22	L. Sibaya	Bruton & Allanson (1974)
" ♀	410	21.7	0.240	5.21	1.99	1.12	2.05	"	"
" ♂	3,082	38.7	0.196	7.59	2.78	1.62	2.47	Incomati Limpopo	Hecht (1980)
" ♀	1,725	30.7	0.246	7.55	2.63	1.55	2.37	"	"
"	655	24.5	0.386	9.46	2.40	1.46	2.36	Njele Dam	"
"	1,729	35.1	0.211	7.41	2.56	1.48	2.41	Winter Dam	"
"	1,671	34.7	0.300	10.41	2.70	1.63	2.56	Loskop Dam	"
"	876	28.0	0.380	10.64	2.52	1.54	2.47	Sheho Ngubu Dam	"
"	1,450	33.0	0.267	8.81	2.59	1.53	2.46	Hartbeespoort Dam	"
"	897	28.2	0.627	17.68	2.75	1.77	2.70	De Hoop Vlei Dam	"
"	1,325	32.0	0.210	6.72	2.44	1.40	2.33	Zeeloei Vlei Dam	"
" ♂	1,259	32.0	0.413	13.22	2.72	1.68	2.63	Loskop Dam	"
" ♀	1,082	28.6	0.397	11.35	2.63	1.62	2.51	"	"
" ♂	770	26.0	0.684	17.78	2.72	1.76	2.67	Doorndrai Dam	"
" ♀	719	25.8	0.438	11.30	2.50	1.55	2.46	"	"
" ♂	737	27.0	0.417	11.26	2.48	1.53	2.48	Luphephe Dam	"
" ♂	675	25.8	0.370	9.55	2.40	1.45	2.39	"	"
"	1,277	31.2	0.644	20.09	2.92	1.88	2.80	Egypt Ponds	Koura & el Bolock (1958)
" ♂	1,979	37.8	0.362	13.68	2.86	1.76	2.71	Hong Kong	Man & Hodgkiss (1977)
" ♀	1,132	31.3	0.395	12.38	2.65	1.63	2.59	"	"
O. andersonii	4,582	48.6	0.174	8.46	2.90	1.68	2.61	Barotse Flats	Duerre (1969)
"	1,826	35.7	0.336	12.00	2.79	1.70	2.63	L. Liambezi	De Merona (1983)
" ♂	1,730	40.2	0.221	8.88	2.58	1.50	2.55	Kafue Flats	Dudley (1974)
" ♀	623	25.1	0.455	11.42	2.45	1.52	2.46	"	"
O. esculentus	744	25.2	0.349	8.79	2.41	1.46	2.35	Zimbabwe	de Kimpe (1965)
"	980	29.5	0.280	8.26	2.44	1.44	2.39	L. Victoria	Garrod (1961)
"	761	26.7	0.450	12.02	2.53	1.57	2.51	"	Lowe (1956)
"	616	25.4	0.529	13.44	2.51	1.58	2.53	"	Trewavas (1984)
"	747	27.1	0.314	8.51	2.37	1.41	2.36	"	"
"	632	25.6	0.307	7.86	2.29	1.36	2.30	"	"
O. leucostictus ♂	581	24.5	1.160	28.42	2.83	1.91	2.84	"	Garrod (1961)
" ♀	449	22.4	0.625	14.00	2.45	1.56	2.50	"	Fryer & Iles (1972)
O. variabilis	426	22.0	0.351	7.72	2.17	1.30	2.23	"	Garrod (1961)
O. saka	663	25.5	0.723	18.44	2.68	1.74	2.67	L. Malawi	De Merona (1983)
O. shiranus	539	24.0	0.499	11.98	2.43	1.52	2.46	"	"
O. squamipinnis	719	26.2	0.448	11.74	2.51	1.56	2.49	"	"
O. niloticus ♂	1,214	33.4	0.233	7.78	2.45	1.42	2.41	L. Alaotra	Moreau (1979)
" ♀	387	22.8	0.439	10.01	2.23	1.37	2.36	"	"
" ♂	851	28.6	0.510	14.58	2.64	1.66	2.62	L. Mantasoa	"
" ♀	746	27.2	0.503	13.68	2.57	1.62	2.57	"	"
" ♀	7,080	57.2	0.137	7.84	2.99	1.70	2.65	L. Itasy	"
" ♀	1,570	34.7	0.275	9.54	2.64	1.57	2.52	"	"
"	1,010	29.4	0.594	17.43	2.78	1.78	2.71	L. Mariout	El Zarka (1961)
"	1,635	34.4	0.450	15.50	2.87	1.80	2.73	"	Payne & Collinson (1983)
"	780	27.1	0.378	10.24	2.47	1.51	2.44	"	"
"	1,013	29.4	0.294	8.64	2.47	1.47	2.41	L. Manzala	"
"	1,373	32.6	0.356	11.61	2.69	1.64	2.58	Nouzha Hydrodrome	Jensen (1967)
"	1,985	36.8	0.283	10.40	2.75	1.65	2.58	L. Tchad	Blache (1964)
"	3,504	39.0	0.500	19.50	3.24	2.06	2.88	L. Albert	Ssentongo (1971)
"	7,134	56.3	0.405	22.80	3.46	2.18	3.11	L. Kainji	Petr & Kapetsky (1983)
"	3,984	46.2	0.546	25.20	3.33	2.13	3.07	L. Nasser	"
"	5,663	52.1	0.216	11.26	3.09	1.84	2.77	"	Payne & Collinson (1983)
T. rendalli ♂	474	22.5	0.667	15.01	2.50	1.61	2.53	L. Itasy	Moreau (1979)
" ♀	434	21.8	0.528	11.51	2.36	1.48	2.40	"	"
" ♂	1,135	30.1	0.253	7.62	2.46	1.44	2.36	L. Alaotra	"
" ♀	610	24.4	0.324	7.91	2.30	1.37	2.29	"	"
" ♂	905	27.2	0.526	14.31	2.68	1.69	2.59	L. Mantasoa	"
" ♀	699	24.9	0.504	12.55	2.55	1.60	2.49	"	"
"	996	29.1	0.745	21.68	2.87	1.87	2.80	Barotse Flats	Duerre (1969)
" ♂	574	24.3	0.479	11.64	2.44	1.52	2.45	"	"
" ♀	2,026	36.9	0.128	4.72	2.41	1.31	2.24	"	"
" ♀	859	27.8	0.467	12.98	2.60	1.63	2.56	Kafue Flats	Dudley (1974)
" ♀	764	26.3	0.456	11.99	2.54	1.58	2.50	"	"
"	2,803	40.0	0.138	5.52	2.59	1.44	2.34	L. Kariba	Balon & Coche (1974)
" ♂	744	26.5	0.313	8.29	2.37	1.41	2.34	Doorndrai Dam	De Merona (1983)
" ♀	2,598	40.2	0.177	7.12	2.66	1.52	2.46	"	"
" ♂	2,766	41.1	0.157	6.45	2.64	1.49	2.42	Incomati Limpopo	De Merona (1983)
" ♀	1,487	33.1	0.230	7.61	2.53	1.48	2.40	"	"
T. zillii ♂	610	24.8	0.538	13.34	2.52	1.59	2.52	River Niger	De Merona (1983)
" ♀	405	21.9	0.603	13.21	2.39	1.52	2.46	"	"
"	832	27.4	0.632	17.32	2.72	1.75	2.68	L. Kainji	Petr & Kapetsky (1983)
"	482	25.5	0.720	18.36	2.54	1.65	2.67	"	"
"	485	23.1	0.334	7.72	2.21	1.31	2.25	L. Mariout	Jensen (1957)
"	196	17.0	0.660	11.22	2.11	1.38	2.28	L. Qarun	Payne & Collinson (1983)
"	274	18.9	0.385	7.28	2.02	1.21	2.14	L. Beteha	El Bolock & Koura (1961)
"	140	15.5	0.510	7.91	1.85	1.16	2.09	Ponds, Egypt	Ben Tuvia (1960)
S. galilaeus ♂	1,126	31.0	0.465	14.42	2.72	1.70	2.65	L. Kinneret	"
" ♀	888	28.9	0.490	14.16	2.64	1.66	2.61	"	"
"	1,262	29.9	0.337	10.08	2.63	1.60	2.48	L. Tchad	Lauzanne (1979)
"	493	23.2	0.670	15.54	2.52	1.62	2.56	L. Kinneret	Landau (1983)
"	506	24.2	0.976	23.59	2.69	1.79	2.76	L. Mariout	Jensen (1957)
"	888	26.6	0.601	15.99	2.73	1.74	2.63	L. Tchad	Blache (1964)
"	811	27.2	0.660	17.95	2.73	1.76	2.69	Chari Logone	De Merona et al. (in press)
"	1,136	30.5	0.530	16.17	2.78	1.76	2.69	L. Beteha	El Bolock & Koura (1961)
"	992	28.5	0.640	18.37	2.73	1.73	2.84	L. Kinneret	"
"	326	20.1	0.530	10.67	2.24	1.40	2.33	L. Manzala	Payne & Collinson (1983)
"	2,750	41.0	0.288	11.81	2.90	1.76	2.68	L. Nasser	Petr & Kapetsky (1983)
"	4,563	48.4	0.469	22.70	3.33	2.11	3.04	L. Kainji	"

Table 4. Indices of growth performance in the six best represented tilapia species in Table 3, ranked according to their mean value of ϕ'. Note that $\overline{\phi}'$ has, overall, the smallest C.V. (in brackets).

Species	n	$\overline{w}$	$\overline{P}$	$\overline{\phi}$	$\overline{\phi}'$
O. niloticus	16	13.5 (39.4%)	2.80 (12.5%)	1.72 (14.2%)	2.65 (8.4%)
S. galilaeus	12	15.7 (26.7%)	2.72 (9.2%)	1.72 (9.5%)	2.65 (6.3%)
O. mossambicus	20	11.0 (35.9%)	2.57 (8.7%)	1.57 (11.2%)	2.48 (7.1%)
T. rendalli	16	10.4 (42.1%)	2.53 (5.7%)	1.53 (8.9%)	2.45 (5.5%)
O. macrochir	8	10.2 (8.9%)	2.42 (5.8%)	1.48 (4.5%)	2.41 (2.8%)
T. zillii	8	12.0 (35.8%)	2.30 (12.6%)	1.44 (15.0%)	2.39 (9.6%)
Mean C.V. (weighted by n)		33.8%	9.04%	10.8%	6.74%

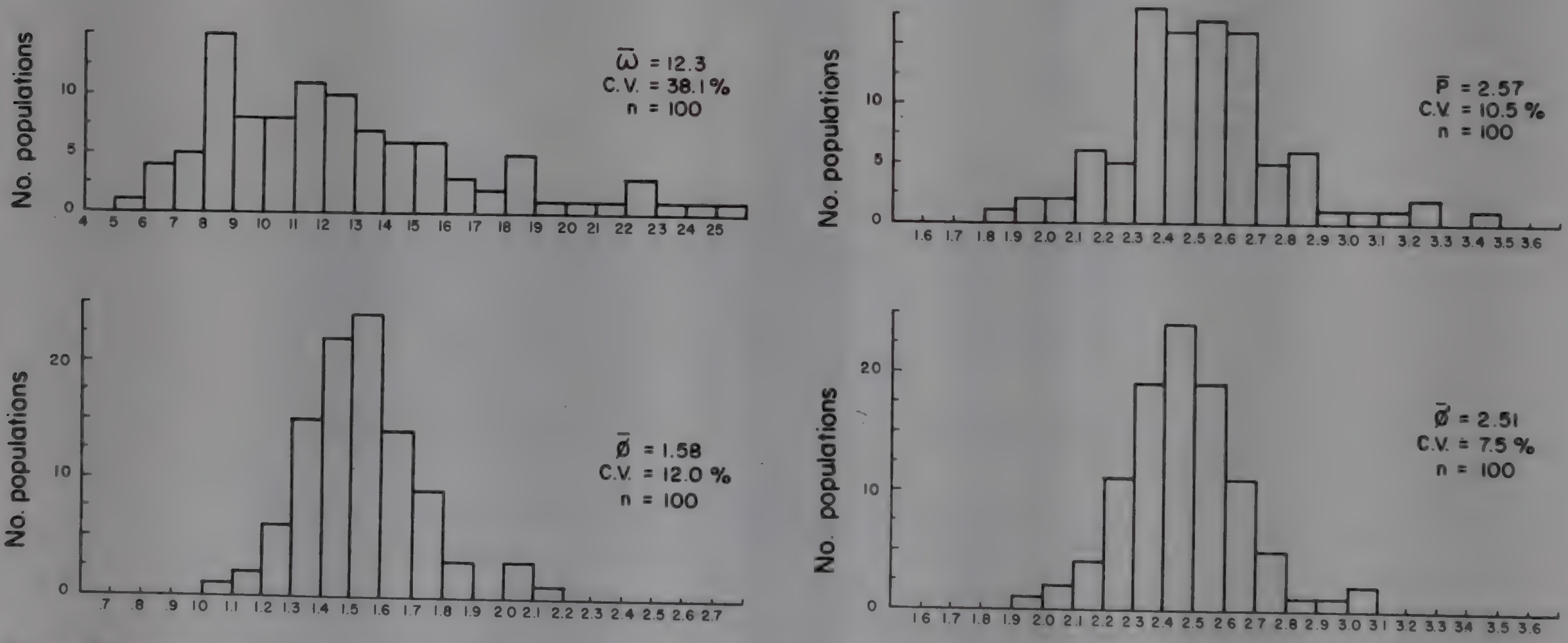

Fig. 1. Frequency distribution of the four indices of growth performance in Table 1. Note low C.V. and correspondingly narrow, sharply peaked distribution of ϕ'.

Distribution and Feeding Ecology of *Penaeus monodon* Along the Coast of Tungkang, Taiwan[1]

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Abstract

The distribution, size composition and feeding ecology of *Penaeus monodon* in the coastal waters of Tungkang, Taiwan, were studied from July 1982 to August 1985.

Gravid female shrimp are found inshore and offshore at depths between 10 and 40 m from June to December. Spawning peaks occur in August and November. Juveniles and subadults are found in the Kaoping, the Tungkang and the Linpien estuaries from March to May. Emigration of subadults from Dapong Bay occurs mainly from June to October. Subadults and adults are caught in appreciable numbers in coastal waters between Dapong Bay and Nanshufu at depths less than 40 m from June to January with the peak from July to October. The stomach contents of the shrimp are mainly composed of crustacea, mollusca, fish, detritus and sand granules. Seasonal and geographical variations in stomach content compositions are significant.

Introduction

To establish an effective system of shrimp ranching fisheries in Taiwan, the Tungkang Marine Laboratory has selected the shrimp fishing grounds along the coast of Tungkang as the experimental area and engaged in a series of studies on the biology, ecology and population dynamics of the economically-important shrimp in this area.

In this paper, the distribution, size composition and feeding ecology of *P. monodon* in the coastal area of Tungkang are presented.

Materials and Methods

The study area includes the Tungkang coast and its adjacent estuaries and bays (Fig. 1).

The catch and effort data were collected from a commercial beam trawler (5.5 t, 22 hp) operated in the coastal area from July 1982 to July 1985. The methods of data collection and processing are the same as those described by Su and Liao (1984). The data of three years were summed up by month and by sampling site and catch per unit effort (CPUE) was calculated.

To assess shrimp in estuaries, a motor raft was employed. A 4 m wide beam trawl fitted with 20-mm mesh net was towed in daytime at a speed about 20 m/min. over the bottom. Each month, five 20-min. trawls were made at the Kaoping, Tungkang and Linpien estuaries from September 1984 to August 1985.

To estimate the emigration of shrimp from the bay into the sea, a sample was taken from a set net at the mouth of Dapong Bay four times every month from September 1984 to August 1985.

In addition to the catch and effort data, monthly samples were collected from the various sampling sites from September 1984 to August 1985. The carapace length (CL) of each specimen was measured to the nearest 0.1 mm, and the body weight (BW) to the nearest 0.01 g. Individual sex was determined and in the case of females a visual estimate of ovarian development was recorded. Stomach contents were preserved in 5% formalin. The fullness of each stomach was determined visually and an index of 0-4 was given corresponding to the quantitative range of empty to full. For stomach content analysis, the entire contents of each stomach were rinsed into a petri dish and examined under a binocular microscope. The frequency of occurrence method (Hyslop 1980) was then employed to elucidate on the spectrum of food ingested.

Results

The monthly distribution of gravid females (spawners) in the coastal waters is shown in Fig. 2. The spawners were absent from the sampling area from January to May. They were caught in the waters between the estuary of Linpien River and Nanshufu at depths of 10-40 m from June to December with the peaks in August and November. CPUEs of 7-8 spawners per ten hours occurred in the peak season.

Fig. 3 shows the monthly distribution of shrimp (excluding spawners) in the coastal waters. No shrimp were caught between Chungjou and Fangliao at depths more than 20 m from February to May. The shrimp occurred between Chungjou and Nanshufu at depths less than 40 m from June to January. Shrimp were more abundant from February to May in the Kaoping, Tungkang and Linpien estuaries and in August in the Tungkang estuary (Fig. 4). The catch data collected at the mouth of Dapong Bay revealed that the shrimp emigrate from the bay to the sea from June to October (Fig. 4).

The size composition of carapace length (CL) by area is shown in Fig. 5. These results show the broad size ranges of shrimp residing in the sampled estuaries and of shrimp emigrating from the Dapong Bay to the sea. The largest shrimp were in general found in the deeper offshore waters.

The intensity of feeding is assumed to be high when the fullness index of stomach is 3 or 4 (50-75% and 75-100% full, respectively). As shown in Table 1, the shrimp in the Dapong Bay forage more intensively in winter and spring. However, in coastal waters intensive feeding occurs in spring and summer.

The stomach contents were classified into eight categories. The relative importance of stomach content by season and by locality is shown in Table 2. In Dapong Bay, Crustacea, Mollusca, fish, detritus and sand granules are the main food. Crustacea occur more often in summer, Mollusca and detritus in spring and sand granules in winter, respectively. Seasonal variation of foraging on fish is not apparent. In coastal waters, Crustacea, Mollusca, detritus and sand granules constitute the main diets. The shrimp forage more often on Crustacea in summer and autumn, Mollusca and detritus in spring and summer and sand grand granules in spring, summer and winter.

Discussion

Judging from the temporal and spatial distribution of CPUEs of spawners (Fig. 2), it is apparent that the main spawning grounds are located at depths from 10 to 40 m to the southwest of the coast between the estuary of Linpien River and Nanshufu. The spawning season is from June to December with peaks in August and November. Motoh (1981) found that the spawners of *P. monodon* could be caught at the mouth of Batan Bay and in the waters off Tigbauan in the Philippines all year-round, but that spawning peaks occur in February, July and November at Batan Bay and in March and October at Tigbauan. Therefore, it is reasonable to conclude that *P. monodon* spawns inshore or offshore depending on favorable salinity and temperature. The spawning season of *P. monodon* in Taiwan is different from the season in the Philippines. Peak spawning takes place in February and March in the Philippines, but not in Taiwan where spawners can rarely be caught from January to May. This difference may be ascribed to geographical variation or overfishing. Local fishermen remember that several years ago spawners of *P. monodon* could be caught in greater numbers from January to May. Urgent action is required to stop the overfishing of *P. monodon* in Taiwan.

Although no shrimp were caught at more than 20 m from February to May (Fig. 3), shrimp were present in estuaries from February to May (Fig. 4). Adolescent and subadult shrimp may be found in nearshore waters at less than 10 m from February to May, although no sampling of these waters was done during this study.

The life cycle of *P. monodon* in the coastal waters of Tungkang is summarized in Fig. 6. Spawning occurs in inshore or offshore waters at depths from 10 to 40 m from June to December. The developing larvae gradually move toward the nearshore waters, estuaries and bays where they grow into adolescents or subadults. After 4-6 months in the natural nurseries, the shrimp swim to deeper water and grow into adults, the mature shrimp at first residing in inshore waters, then moving offshore gradually.

Marte (1980) studied the food and feeding habit of *P. monodon* from Makato River, Philippines, and reported that they feed mainly on Crustacea and Mollusca. Organic detritus, silt and sand were ingested in relatively small amounts. However, this study found that Crustacea, Mollusca, detritus and sand granules are all ingested intensively. The seasonal variations in feeding intensity (Table 1) and stomach contents (Table 2) suggest that the food environment changes with the seasons. Fish fragments are found in the stomach contents of the shrimp from the bay, but not in those from the sea (Table 2). This suggests that their feeding habit is modified by food environment.

The results of this study suggest that *P. monodon* is a suitable species for sea ranching in the coastal waters of Tungkang. The juvenile, adolescent and subadult shrimp reside in the estuaries, the bay and the nearshore waters. The subadults and adults are largely distributed in inshore waters. Shrimp foods are abundant in coastal waters. Released shrimp can have sufficient food for growing and can be harvested from specific areas. In fact, several trial releases of tagged *P. monodon* (50 g BW in average) have been done, with average recovery rates of 15% and maximum growth of about 30 g/month (Su and Liao, unpublished data). It must be noted, however, that unless controls on fry fishing in bays and estuaries are implemented, these waters cannot be used as nurseries. These preliminary results suggest that the future of *P. monodon* ranching in this area is quite promising.

Acknowledgements

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Mr. Adam Body for reading the manuscript. The research project was supported by the National Science Council (NSC 74-0409-B056-05).

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Table 1. Fullness of stomachs of *P. monodon* by season and locality (%).

Fullness index*	Winter (Jan-Mar) A	B	Spring (Apr-Jun) A	B	Summer (Jul-Sep) A	B	Autumn (Oct-Dec) A	B
0	17	7	43	0	40	1	43	3
1	33	69	20	52	33	25	27	50
2	16	15	8	14	16	14	11	17
3	17	7	15	5	6	19	9	16
4	17	2	14	29	5	42	11	14
No. of stomachs examined	36	13	49	21	221	144	301	78

*Index 0, 1, 2, 3, 4 indicate: empty, 1-25%, 26-50%, 50-75%, 75-100% fullness, respectively.

A: Dapong Bay; B: Coastal waters.

Table 2. Occurrence of stomach contents of *P. monodon* by season and locality (%).

Stomach contents	Winter (Jan-Mar) A	B	Spring (Apr-Jun) A	B	Summer (Jul-Sep) A	B	Autumn (Oct-Dec) A	B
Crustacea	63.6	8.3	57.1	24.8	75.2	58.6	53.8	48.2
Mollusca	40.9	33.3	57.1	61.9	40.9	56.9	42.4	28.3
Fish	27.3	0	36.7	0	30.5	4.8	28.0	5.6
Detritus	68.2	75.0	82.1	100	67.6	91.8	62.9	83.0
Sand granules	77.3	75.0	71.4	95.2	66.7	98.3	46.9	92.4
Plant material	0	0	0	0	0	6.5	0	0
Radiolarians	0	0	0	14.2	0	19.5	0	1.8
Sponges	0	0	0	0	0	6.5	0	0
No. of stomachs* examined	22	12	28	21	106	123	132	53

*With fullness index 3 or 4.
A: Dapong Bay; B: Coastal waters.

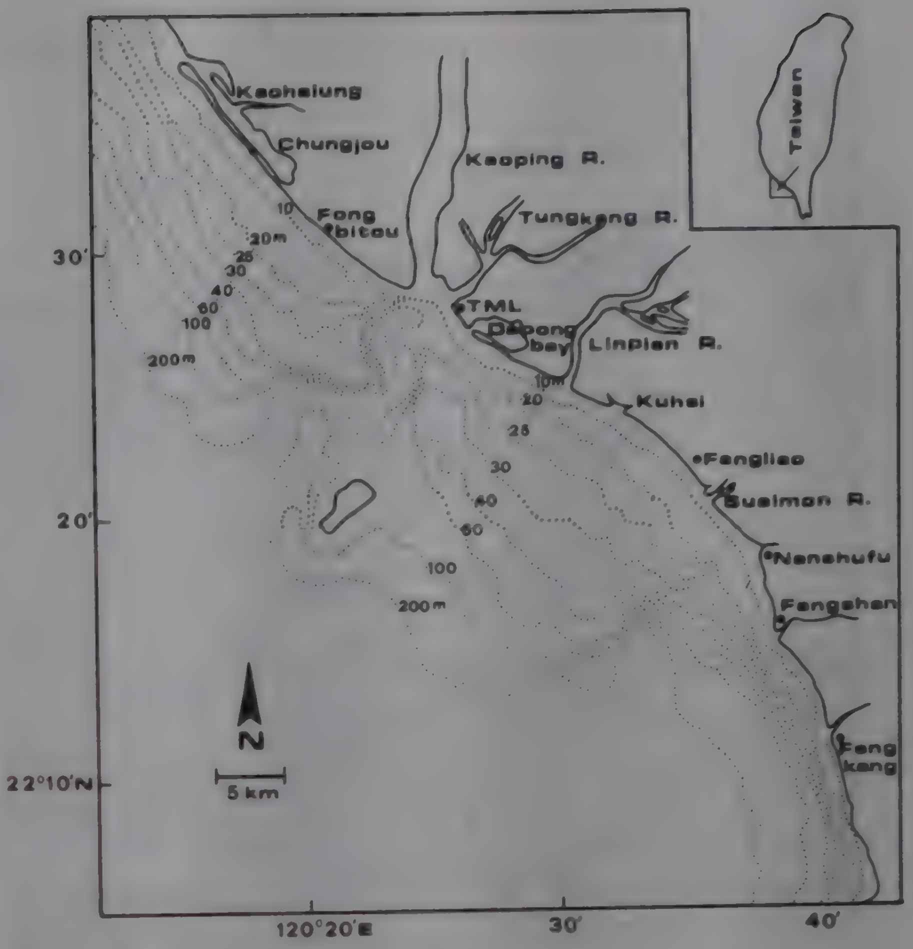
Kaohsiung
Chungjou
Kaoping R.
Tungkang R.
Fong bitou
TML
Dapong bay
Linpien R.
Kuhsi
Fangliao
Suaiman R.
Nanshufu
Fengshan
Fong kang
Taiwan
10
20m
25
30
40
60
100
200m
10m
20
25
30
40
50
100
200m
N
5 km
30'
20'
22°10'N
120°20'E
30'
40'

Fig. 1. Map showing the study area.

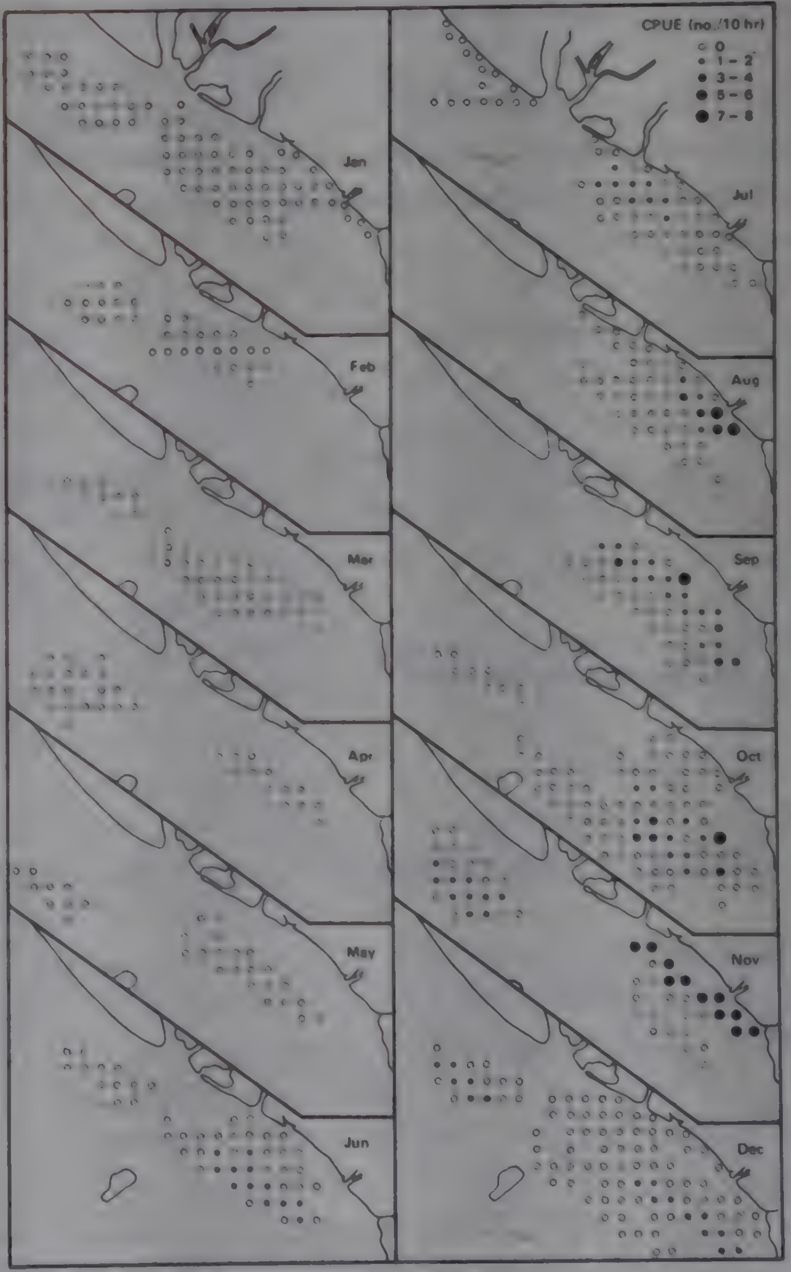
CPUE (no./10 hr)
○ 0
● 1 - 2
● 3 - 4
● 5 - 6
● 7 - 8
Jan
Feb
Mar
Apr
May
Jun
Jul
Aug
Sep
Oct
Nov
Dec

Fig. 2. Monthly distribution of CPUE (in number per 10 hours) of spawners of *P. monodon*.

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[1] Contribution A No. 61 from the Tungkang Marine Laboratory.

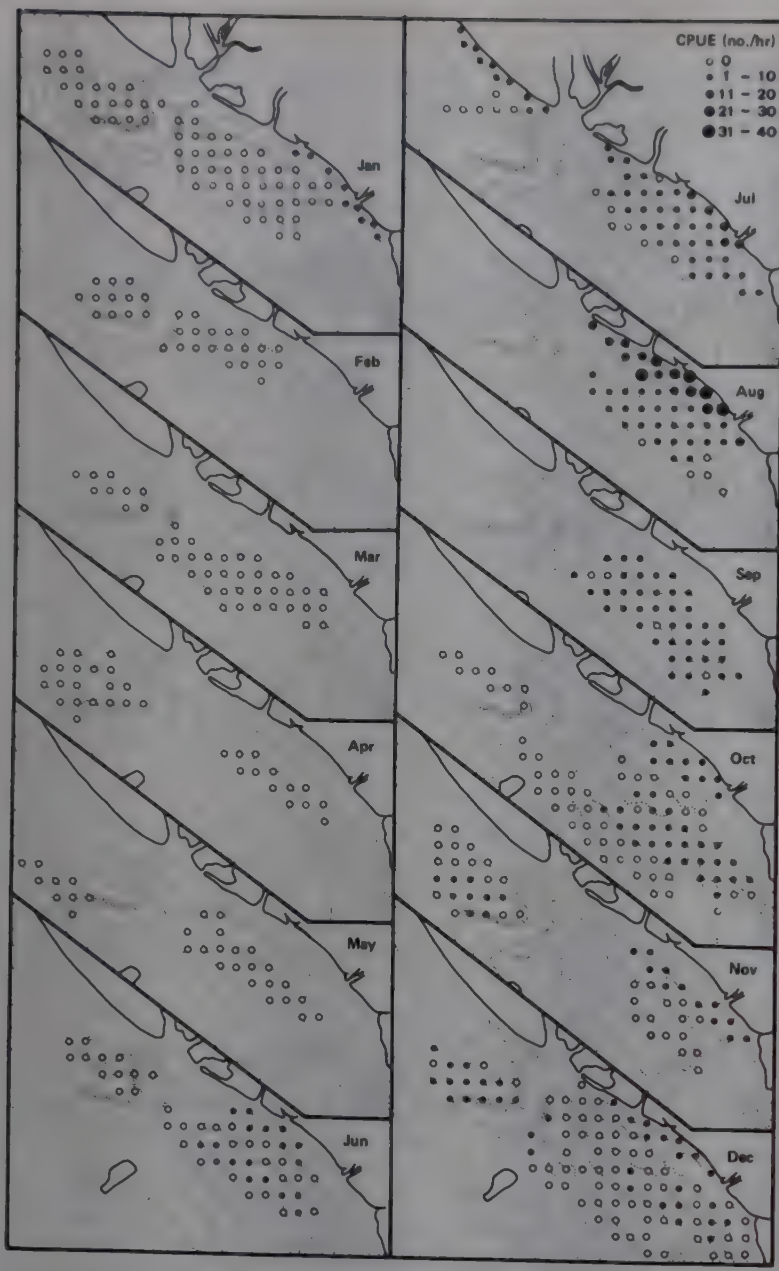
CPUE (no./hr)
○ 0
• 1 – 10
• 11 – 20
● 21 – 30
● 31 – 40
Jan
Feb
Mar
Apr
May
Jun
Jul
Aug
Sep
Oct
Nov
Dec

Fig. 3. Monthly distribution of CPUE (in number per hour) of *P. monodon*.

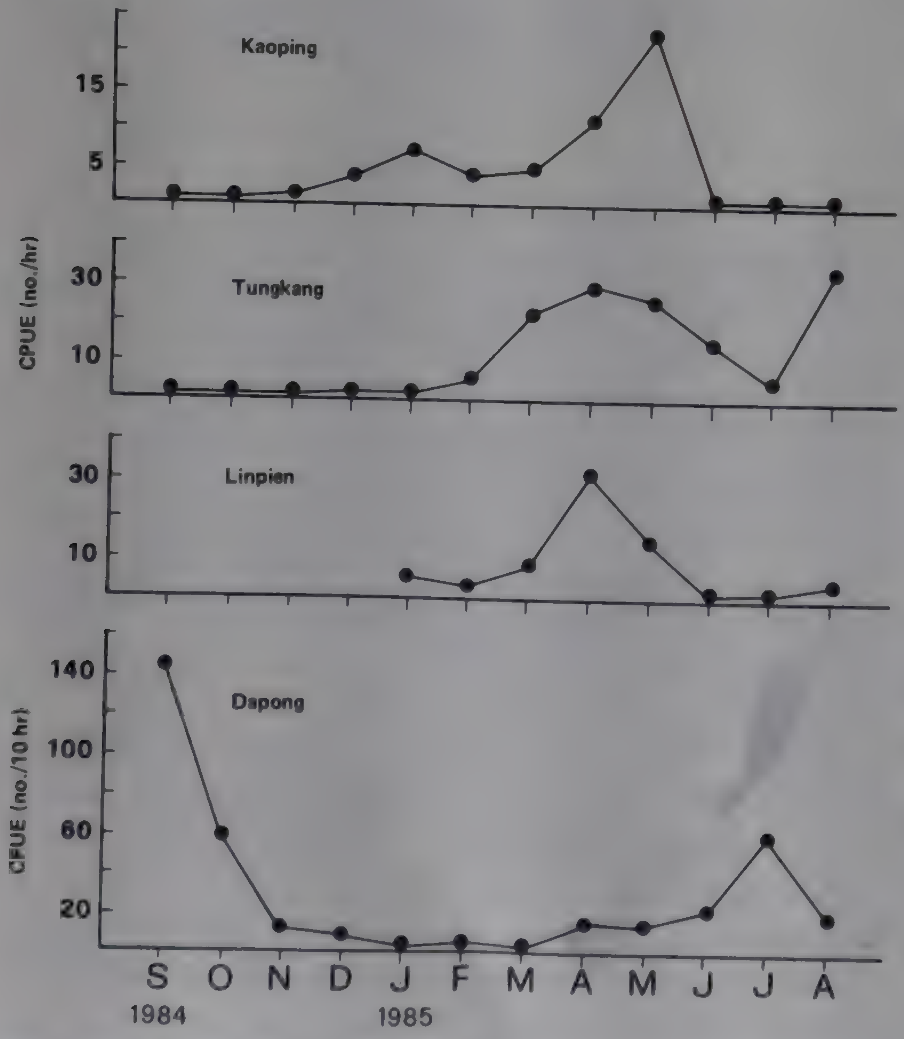

Fig. 4. Monthly occurrence of *P. monodon* at various sampling sites.

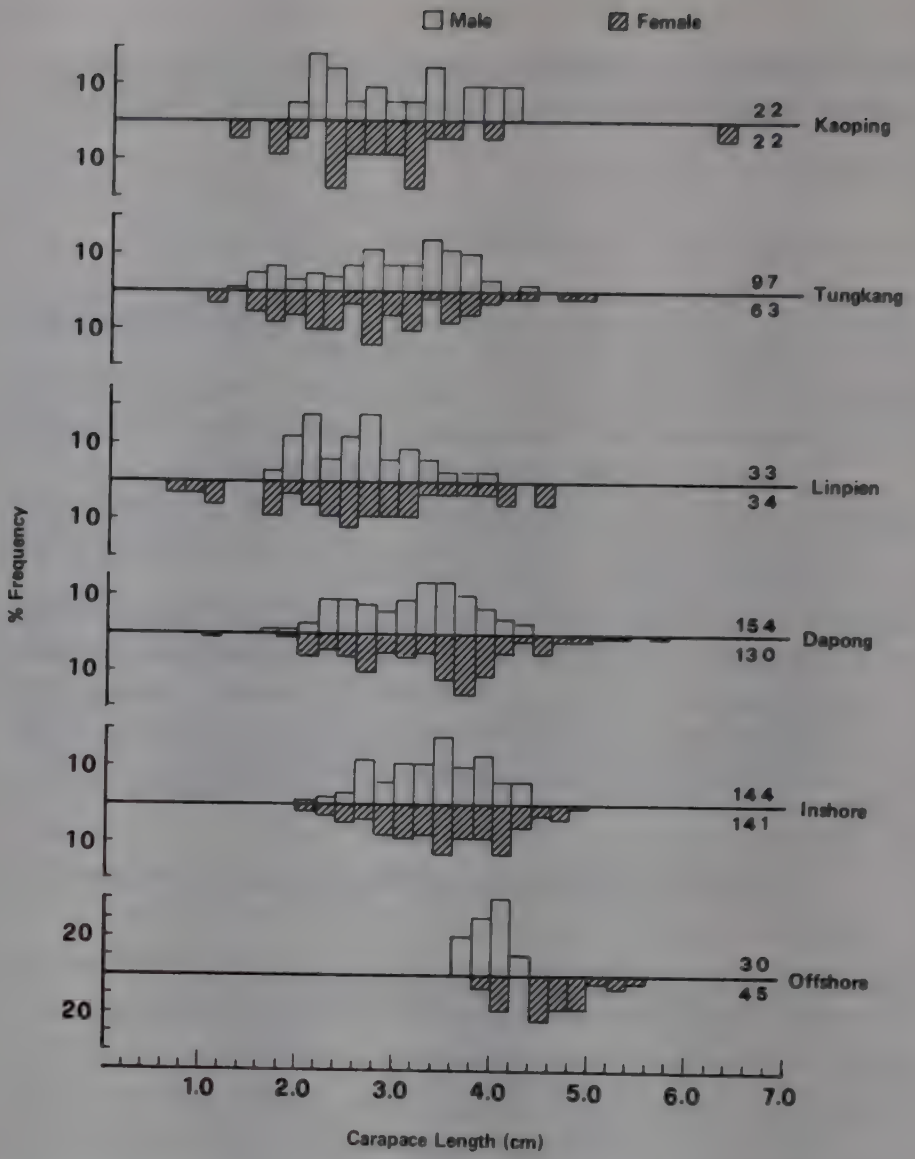

Fig. 5. Carapace length frequency distribution of *P. monodon* by area and sex. The number in the figure indicates sample size.

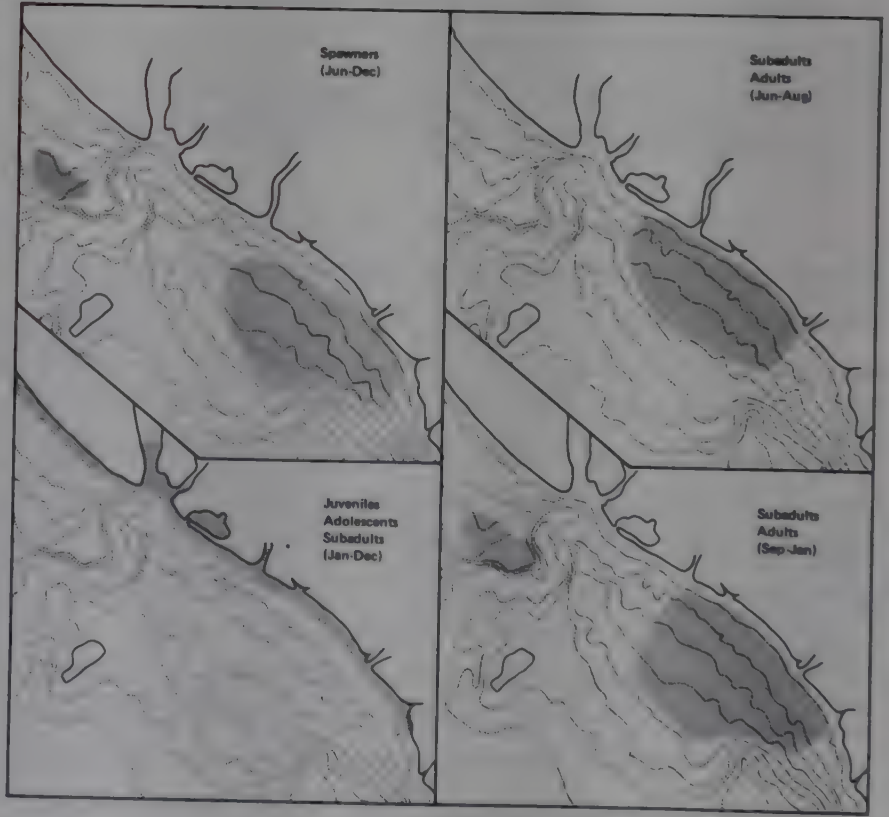
Spawners (Jun-Dec)
Subadults Adults (Jun-Aug)
Juveniles Adolescents Subadults (Jan-Dec)
Subadults Adults (Sep-Jan)

Fig. 6. Schematic figure of life cycle for *P. monodon* in the coastal waters of Tungkang. The shaded area indicates the range of distribution.

The Biology and Culture of *Ranina ranina* Linnaeus

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Abstract

The biology and culture of the spanner crab, *Ranina ranina* Linnaeus, was studied for a 14-month period in Jolo, Sulu and Camiguin Island, Misamis Oriental, Philippines. Two stations were established in Jolo and two in Camiguin Island. *R. ranina* sampling was done by skin diving and baited crab traps known as benthol. Monthly ecological sampling for plankton, meiofauna, physicochemical parameters and commercial catch was undertaken. Collected adult and berried *R. ranina* were reared under laboratory conditions in wooden aquaria, fiberglass and concrete tanks. Observations revealed that *R. ranina* thrives in coralline sand with depths ranging from 3.2 to 100 m. Plankton and meiofauna communities in all stations showed similar taxa composition. Commercial fishing was observed to be well-developed and organized in Jolo, Sulu. Transport of live male, female and berried specimens yielded 100% survival with the use of aerated or oxygenated seawater. Berried females hatched 80,000-160,000 zoeae. Feeding requirement studies indicated that the zoeae survived significantly longer with *Brachionus* feeding. *R. ranina* broodstock spawned in captivity with or without eyestalk extirpation at 1:1 sex ratio and fed with squid and shark meat.

Introduction

The biology and culture of *Ranina ranina* Linnaeus, known as spanner crab or frog crab (Australia and USA) or kona crab (Hawaii), *kuratsa* (Zamboanga), *kayukom* (Camiguin), *bayukom* (Camiguin) or *kagang pama* (Jolo and Tawi-Tawi), are important for investigation considering its contribution to the protein needs of the country and its economic prospects in the local and export markets. Its biology and culture are practically unknown.

The special characteristics of spanner crab were studied to establish benchmark information on the ecology and rearing of larvae, juveniles and adults. Initial results obtained from October 1981 to December 1982, with notes on spanner crabs in general are presented.

Materials and Methods

Fig. 1 shows the established stations and substations in Jolo, Sulu and Camiguin Island, Misamis Oriental, Philippines. Sampling was done in all stations by using baited crab traps (Fig. 2) known as *sambao* (Jolo and Tawi-Tawi) or *benthol* and *Sapyaw* (Camiguin) and by skin diving every three days before the first (Stations 1-2) and last (Stations 3-4) quarters of the moon.

Samples were transported to the laboratory by land and water. The specimens were placed in plastic pails with unaerated seawater or plastic bags with oxygenated seawater encased in wicker bags.

Plankton samples were obtained in all stations with a Kittahara quantitative plankton net to examine the relative abundance of crab planktonic larval species and to identify and quantify *R. ranina* larvae. Meiofauna cores of sediment, both from deep and shallow water, were obtained to determine the interstitial organisms and interstitial water and organic carbon content (Tietjen 1969; Hulings and Gray 1971) of the substrate where *R. ranina* burrow. Food types and feeding habits were also determined. Carapace length-width and body weight were measured. Water temperature was recorded and pH, turbidity, salinity, dissolved oxygen, NO_3, NH_4 and PO_4 were analyzed by standard methods (Theoroux et al. 1943; Strickland and Parsons 1972). Grain-size analysis determined the substrate types in all stations.

Interview and market surveys were conducted to assess spanner crab landings. Fishing operations were also observed to determine the composition of the catch per month.

Laboratory operations were done with different life stages on different diets in three different setups with five treatments and replicates in concrete tanks, wooden aquarium and fiberglass tanks.

Broodstock development and management techniques were applied to raise spawners in captivity. Rearing schemes on larvae and adults were modified from Provenzano (1967). Water quality, feeding ration and requirements, pests, parasites, stocking density and sex ratio were monitored. Tests on broodstock are still in progress.

Results and Discussion

The specimens collected exhibited characteristics of *R. ranina* (Sakai 1976), being of large size with very broad carapace (6:5 ratio), sternal thoracic shield reaching only to the bases of the ambulatory legs, and three-segmented eyestalk with all four pairs similar in form and size.

The crabs examined have elongated mouth parts with triangular buccal frame, an adaptation to a back-burrowing habit. The narrow upper end of the buccal frame and the elongated maxillipeds function as exhalant channel. Its elongated body and spade shaped legs are convenient for digging, burrowing or swimming. Underwater on coralline sandy substratum, crabs were observed to burrow by tilting backwards and to dig with their walking legs. Burrowing is rapid until only the eyes or antennae are invisible. In culture tanks they exhibited the same behavior but were not aggressive and could be easily handled without danger or biting.

The three-month survey during the 1981 and 1982 seasons showed that the animal occurred in comparative abundance in Maimbung, Jolo, Sulu (Table 1).

Data from interviews and *sambao* operations with fishermen on fishing gear components and fishing time and effort are presented in Tables 2 and 3.

Observations on holding time after collection by *benthol* in Station 2 showed that 100% survival could be attained with dorsal orientation inside pails or basins with or without substratum but with seawater and aeration from 2 to 24 hours on board a boat (Tables 4 and 5). Survival of crabs transported from study areas to the laboratory with aerated or oxygenated seawater was 100% (Table 6).

Meiofauna analyses revealed the presence of copepods, nematodes, polychaetes, kinorhynchs, decapod larvae, turbellaria, tardigrades, oligochaetes, ostracods and bivalve larvae in all stations. Guts of all crab samples contained foraminiferans and undigestible parts of exuviae of shrimps, crustaceans, polychaetes, copepods, other unrecognizable materials and sand grains. Parrot fish were the only predators observed.

Maimbung, Jolo, Sulu has a comparatively well-developed and better organized fishery. Although the fishery already existed in Jolo and Tawi-tawi in the early 1950s it was only in 1976 that the fishery converged in Maimbung and became organized. Catches from Maimbung Bay, Siasi Island, Tapol Island and Kuliasi are arranged in bundles of 2-9 pieces of 500-1,000 g and sold at ₱4.00/bundle to middlemen in Maimbung. These are then transported to Jolo market at ₱2-15/piece. or at ₱8-10/bundle depending on the size. Those taken to Zamboanga and Manila are packed in styrofoam boxes with crushed ice and sold at ₱20/kg or ₱5-20/piece depending on the size.

Hatchery operations were first attempted with a gravid female from Medano Islet, Camiguin Island, Misamis Oriental. The gravid specimen initially spawned 160,000 zoeae which lived 12 days after hatching. This spent female spawned the second time; 80,000 zoeae hatched one month later and lived up to 19 days on *Brachionus* spp. alone. Another experiment with *Brachionus* sp., *Dunaliella* sp., clam juice, eggyolk and Diet III juice was done in five treatments and five replicates. A highly significant difference was obtained with *Brachionus* sp. feeding alone at 100 *Brachionus* sp. individuals/zoeae and feeding with *Brachionus* sp., *Dunaliella* sp. and eggyolk against clam juice and Diet III juice. Mortality was attributed to the presence of *Legenidium* sp.

In wooden aquaria with the use of aquarium jackets with lights to maintain water temperature and *Artemia*, *Brachionus*, *Tetraselmis* and diatoms as feeds, crab larvae survived up to 14 days.

In the 1982 hatchery operation, a gravid female from Jolo, Sulu, died upon extrusion of the first batch of eggs. This was attributed to the stress encountered by the berried females during transport. The zoeae were reared in chlorinated water with *Artemia*, *Brachionus*, eggyolk and diatoms and survived to the 16th day.

In the 1982 operations, observations were made on the sexual maturity of extirpated adults taken from Jolo, Sulu and Camiguin Island. These were cultured in wooden aquaria at 1:1 ratio. During fertilization, the male was observed clasping the female in upright position using the sandy substratum as support. The premating embrace took place 5-7 days before the copulatory molt and lasted for about three to four days. The pair separated when the female was on the verge of pre-copulatory molt. A few hours after molting, the male embraced the female again for the actual mating. The initial 14 pairs of broodstock at 1:1 sex ratio yielded four berried females in captivity. Two aborted their eggs, one spawned and one died. Broodstock development studies in 1-t fiberglass tanks and 18-t concrete tanks are still in progress.

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Table 1. Total catch (no. crabs) of designated crab landing areas for three months of 1981 and 1982.

	1981				1982			
Months	1	2	3	4	1	2	3	4
June	15	119	5	12	20	305	4	7
October	16	360	6	2	15	200	18	5
November	10	200	10	4	12	400	8	10

Table 2. Fishing gear component and fishing effort in designated areas.

Areas	Depth (m)	*Bentol*/operation (no.)	Operation time (hr)	Bait	Catch/6 hr or /dive
Zamboanga	32 – 64	9	5:30 – 11:30 a.m.	Shark	1 – 4
Basilan	32 – 64	9	5:30 – 11:30 a.m.	Shark	1 – 4
Patikul	32 – 64	28	5:30 – 11:30 a.m.	Rayfish	4 – 12
Maimbung	48 – 100	45	5:30 – 11:30 a.m.	Shark	12 – 60
Medano Is.	32 – 64	9	6:00 – 3:00 p.m.	Surgeon fish	0 – 7
Medano Is.	3.2 – 16	Skin dive	6:00 – 3:00 p.m.	–	1 – 2/dive
Magting	3.2 – 16	Skin dive	6:00 – 3:00 p.m.	–	1 – 2/dive
			3:00 – 5:00 p.m.	–	3 – 25

Table 3. Biological composition of catch by *bentol* and diving in July, August and September, 1982.

Station	No. of observations	Total catch (/6 hr)	Biological composition Male	 Female	 Female with eggs	WW* ranges (g)	$\bar{x}$ WW (g)
1	1	12	5	6	0	100 – 600	225
	2	4	2	2	0	110 – 160	130
	3	9	4	5	0	75 – 130	100
2	1	15	6	9	1	150 – 775	320
	2	9	4	5	5	200 – 500	367
	3	16	8	8	8	100 – 700	281
3	1	6	3	3	0	63 – 116	94
	2	9	5	4	0	140 – 410	253
	3	13	7	0	0	105 – 398	206

*WW = wet weight.

Table 4. Survival of males and females in holding facilities on a boat. Values are means of duplicate setups.

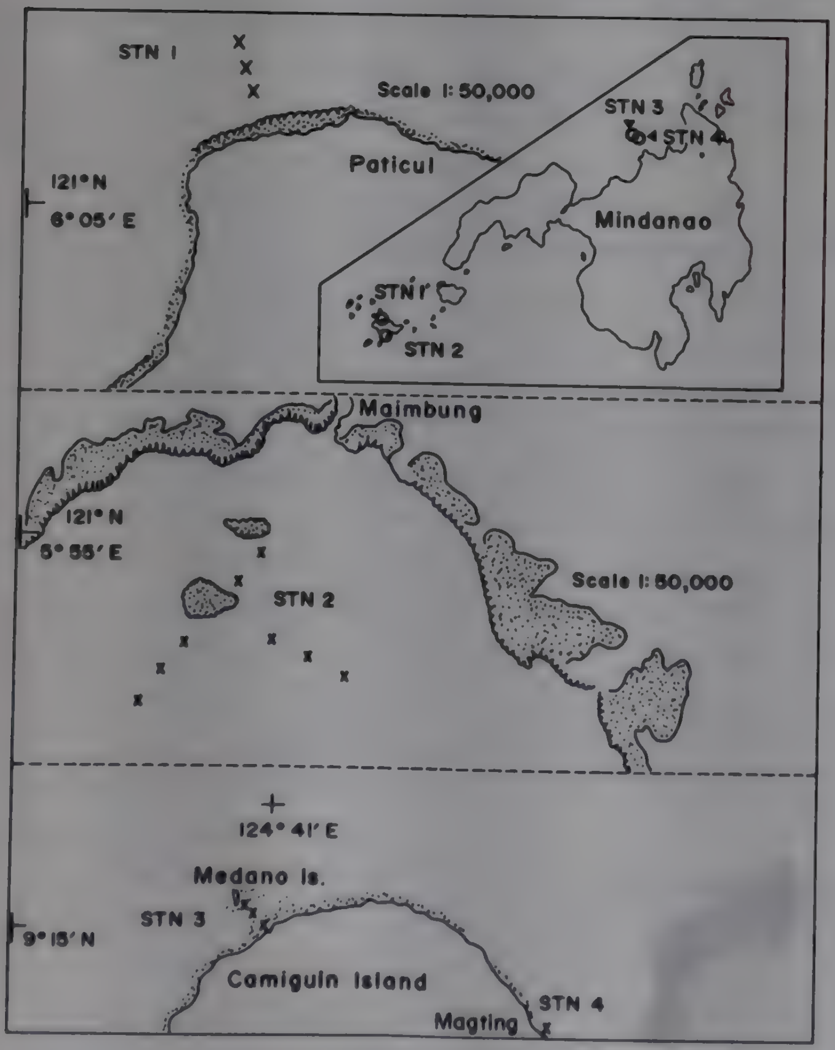
Treatment	Position and medium	No./ basin	Sex ratio	Holding time (hr)	Survival (%)
1	ventral, without seawater	8	1:1	5	0
2	dorsal, without seawater	8	1:1	12	80
3	dorsal, without substrate and aerated seawater	8	1:1	24	100
4	dorsal, without substrate but with aerated seawater	8	1:1	24	100

Table 5. Survival of male and female *R. ranina* in holding facilities of *banca*. Values are means of duplicate setups.

Treatment	Medium	No./ pail	Sex ratio	Holding time (hr)	Survival (%)
1	19 l seawater	4	1:1	2	100
2	19 l seawater	4	1:1	6	100
3	19 l seawater	4	1:1	8	100
4	19 l 2/aeration but change water every 2 hr	8	1:1	24	100
5	10 l seawater without aeration	8	1:1	24	0

Table 6. Survival and transport methods and duration from study areas to MSU-IFRD laboratory.

No. of trials	Containers	Transport medium	Density/ bag	Survival (%)
Station 2: 24-28 hr				
3	plastic bags in wicker bags	chilled seawater with oxygen	4 (2♂, 2♀)	100
			4 (2♂, 2♀)	100
			4 (2♂, 2♀)	100
3	plastic bags in wicker bags	chilled seawater with oxygen	6 (5♂, 2♀)	100
			6 (4♂, 1♀)	100
			6 (3♂, 3♀)	100
3	plastic bags in wicker bags	chilled seawater with oxygen	1 (♀ berried)	100
			2 (♀ berried)	100
			1 (♀ berried)	100
Station 3: 8-12 hr				
3	20-l pails	seawater with sediment and with aeration	4 (2♂, 2♀)	100
			4 (2♂, 2♀)	100
			4 (2♂, 2♀)	100
3	20-l pails	seawater without sediment and aeration	4 (2♂, 2♀)	50
			4 (2♂, 2♀)	75
			4 (2♂, 2♀)	100
3	plastic bags in styrofoam box	chilled seawater with oxygen	6 (4♂, 2♀)	100
			6 (3♂, 3♀)	100
			6 (3♂, 3♀)	100
3	plastic bags in styrofoam box	chilled seawater with oxygen	1 (♀ berried)	100
			1 (♀ berried)	100
			1 (♀ berried)	100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STN 3
STN 4
Paticul
121° N
6° 05′ E
Mindanao
STN 1
STN 2
Maimbung
121° N
5° 55′ E
STN 2
Scale 1: 50,000
124° 41′ E
Medano Is.
STN 3
9° 15′ N
Camiguin Island
STN 4
Magting

Fig. 1. Study areas in Paticul, Jolo (Stn. 1); Maimbung, Jolo (Stn. 2); Medano Islet, Camiguin (Stn. 3) and Magting, Camiguin (Stn. 4). STN = station.

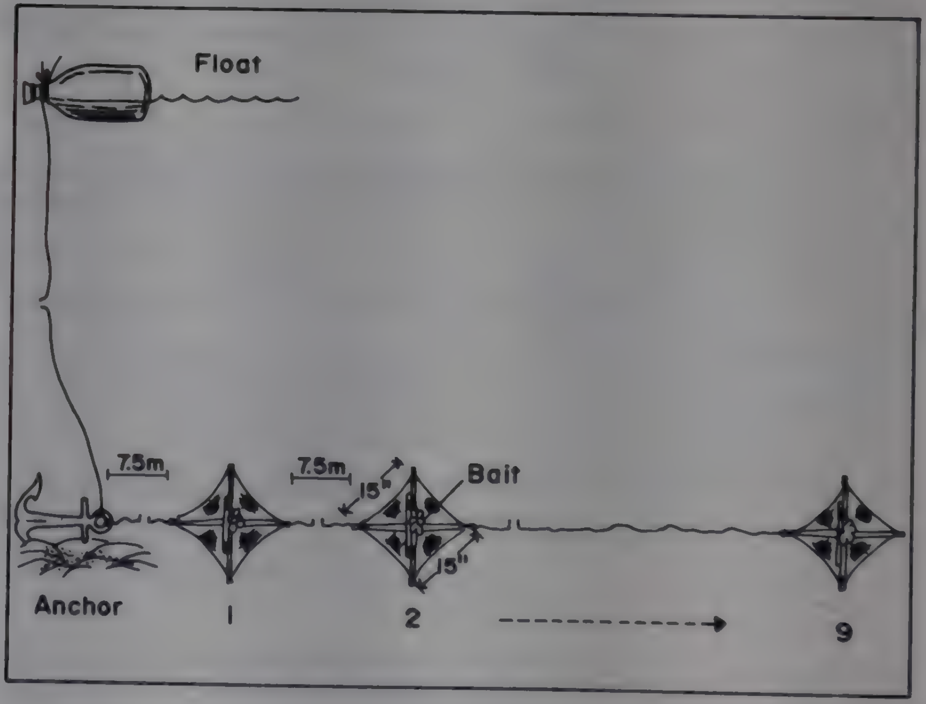
Float
7.5m
7.5m
15"
15"
Bait
Anchor
1
2
9

Fig. 2. Crab fishing with *sambao* (Tausog) or *sapyaw* (Visayan).

Ichthyoplankton Studies in the Southern Java Sea

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Abstract

Ichthyoplankton samples were collected over a one-year period from the southern Java Sea around Jepara, Central Java, Indonesia. Oceanographic data and horizontal ichthyoplankton samples were taken at all 15 sites each month, and vertical samples were also taken monthly at the nine deeper stations (10-30 m). Nearly 9,000 eggs and 1,700 larvae were collected. *Stolephorus* (anchovy) eggs made up the largest identifiable egg category with 7% of the total; and clupeiform larvae were the dominant element of the larval fraction with 42%. In the horizontal samples the maximum egg density at any site was 31.8/m^3, and the annual mean density 0.7, eggs/m^3. The maximum larval density was 1.8/m^3, and the annual mean, 0.1/m^3. In the vertical hauls the maximum egg density was 20.3/m^3 (annual mean 1.9/m^3) and the maximum larval density 31.5/m^3 (annual mean 1.3/m^3). The value of larval surveys covering such limited areas is discussed in relation to fish biology, population dynamics and fishery resource appraisal.

Introduction

Hempel (1973) summarized the uses of ichthyoplankton studies in three categories: detection and appraisal of fisheries resources; studies in population dynamics of fishes; and studies in biology and systematics. The FAO manual on standard techniques for fish egg and larva surveys (Smith and Richardson 1977) gave excellent guidelines on how to conduct such ichthyoplankton studies, but assumed that the boats available would be 15-100 m in length and fully equipped with a complete range of winches and oceanographic equipment.

Is it worthwhile for groups with limited resources to even consider starting such work, and if they do, what aspects of ichthyoplankton studies could they attempt? Many of the uses of ichthyoplankton surveys involve monitoring long-term fluctuations in the success rates of breeding populations, but in most Asian waters even the initial egg and larva surveys and identifications have not yet been done. Until some of this knowledge is obtained from small-scale surveys, larger-scale stock monitoring exercises will have difficulty in functioning effectively.

The objective of this program was to examine the abundance and distribution of the ichthyoplankton in a small coastal area of the southern Java Sea. Particular attention was to be paid to the breeding of anchovies *(Stolephorus* spp.*)* and small clupeids *(Sardinella* and *Dussumieria* spp.*)* which are important in the local fisheries.

The Study Area - The Java Sea

As recently as 12,000 years ago, what is now the Java Sea was a major plain and river valley draining the Sunda Shelf between Kalimantan and northern Java, and emptying into the Straits of Makassar to the east (Tjia 1980). Sea level changes and crustal movements have since produced the Java Sea, which is approximately 1,000 km long and 400 km wide, but no more than 60 m deep for most of its area (Fig. 1a).

The Java Sea lies 4-8^{o} south of the equator in an area which is dominated by relatively strong, wet, northwesterly monsoon winds between November and February; and weaker, drier southeasterly winds between June and September. The monsoon winds set up a current system within the Java Sea. A surface current running from west to east occurs during the northwesterly monsoon, bringing less saline water from the South China Sea into the western part of the Java Sea. This current reverses during the southeasterly monsoon when more saline, oceanic water is brought in from the east (Soegiarto and Birowo 1975; Groves and Niemeyer 1975).

Much of the oceanographic and biological data relating to the Java Sea describes the conditions well away from the coastlines and the immediate influence of land (Wyrtki 1957; Soegiarto and Birowo 1975). This program considered the environment of the southern Java Sea immediately adjacent to the Jepara headland on the north coast of Central Java as far out as the 30 m isobath.

Materials and Methods

The program was based on 15 sampling sites located along three parallel transects situated approximately 10 km apart (Fig. 1b). Samples were taken at monthly intervals in or over water of 2, 5, 10, 20 and 30 m depth along the transect lines. The work was carried out between June 1984 and May 1985 from a 6-m aluminum launch.

Several physical parameters were measured: surface temperatures, salinities and Secchi disc extinction depths. the speeds of surface currents were measured from the anchored boat using a simple rectangular drogue set 2 m below the surface and attached to a measuring cord. Current direction was assessed using a hand-held compass.

Samples were taken using a simple conical plankton net (45-cm mouth diameter) fitted with 500μm mesh and a centrally situated flowmeter. Horizontal surface tows of approximately 5 minutes duration were made at each site, and vertical or near-vertical hauls were made at 10, 20 and 30 m sites on all transects. The samples were stored in 5% buffered formalin until examined. All the fish eggs and larvae were removed from the plankton samples, counted and the numbers converted to counts/m^3 of filtered seawater.

Results and Discussion

The monthly surface temperatures in the sampling area (15 measurements/month) oscillated about an annual mean of 29.3°C (range 27.6-31.0°C), which is considerably higher than that quoted for the central Java Sea by Wyrtki (1957) who found a mean of 28.2°C and a range of 27.6-28.8°C. The form of the seasonal variation found here was as expected during the drier part of the year, when the sea surface temperature rose steadily, but rather than falling steadily throughout the rainy season as expected (Wyrtki 1957; Kastoro and Birowo 1974), there was a large rise in the mean temperature in January to a peak of 31.0°C. Climatological data provided by the Semarang Meteorological Office showed that the sunshine duration for January 1985 was 10% higher than any previous figure in the 10-year range, and 27% above the 10-year average. It is very probable that this would have been sufficient to have caused the unusually high January sea surface temperatures and the raised annual mean temperature.

The mean salinity of the surface waters over the 2-m isobath reached a maximum of 31.9 ppt in September and a minimum of 18.0 ppt during December at the height of the rainy season. Salinities at the 30-m isobath followed the same pattern, but reached a peak of 32.5 ppt in August and fell to 25.6 ppt in December. Salinity in the central Java Sea varies with location, but generally fluctuates between 32 ppt in February/March and 34 ppt in September (Sjarif 1959; Soegiarto and Birowo 1975). Inshore results from work in the western Java Sea (Nontji 1978) and eastern Java Sea (Soegiarto et al. 1980) are similar to those presented here, and confirm the expectation that inshore salinities are more variable and generally lower than offshore.

The mean Secchi disc extinction depths at the inner stations (2, 5 and 10 m) were dominated throughout the year by the effects of coastal wave action and terrestrial run-off, but those over the 20-m and 30-m sampling sites gave results which were initially rather surprising. Between June and November the three 30-m sites gave average extinction depths which varied between 3.8 m and 8.2 m, but starting in December (the northwest monsoon period) the transparency increased dramatically, reaching a peak of 19.0 m in March. The transparencies at the 20-m sites followed the same pattern.

Increases in water transparency may be caused either by decreases in the amount of suspended inorganic matter, or by decreases in phytoplankton abundance. Both major storms and pulses of phytoplankton production occurred during the December-March build-up of high transparency, and as both of these factors would be expected to decrease rather than increase the transparency, they could not have played a major role in producing the observed results.

A third possibility is that water movements caused by the monsoons were responsible for the high transparency. The surface water coming to Jepara in March is derived from the Sunda Shelf to the northwest of the study area (Soegiarto and Birowo 1975). This water will have been travelling across the Java Sea for 2-3 months, rather than along shallow coastal areas, and could therefore be expected to have lost much of its sediment load and be considerably clearer than inshore water.

The surface current data for the area showed two major features: north to northeasterly flowing currents of up to 2 km/hr from December to February; and generally southerly flowing currents seldom reaching 1 km/hr from June to August. These results are in accordance with the wind patterns and theoretical expectations (Groves and Niemeyer 1975) and add some of the fine detail to previous work.

During this work, 8,830 eggs and 1,710 larvae were taken. Within these categories there were more than 30 identifiably different eggs and more than 100 different larvae and juveniles. *Stolephorus* (anchovy) eggs (7%) made up the largest immediately identifiable category of eggs, and clupeiform larvae (42%) were the dominant larvae.

The average densities (15 samples/month) of fish eggs and larvae taken in surface horizontal hauls between June 1984 and May 1985 are shown in Fig. 2. The annual mean density for all eggs was 0.66/m^3, although a peak single-sample density of 31.75/m^3 was recorded over 20 m of water in September. As a result of this sample, September also showed the maximum monthly average egg density of 2.70/m^3. *Stolephorus* eggs averaged only 0.04/m^3 over the year, with a small peak in abundance in April. These egg densities are about three times the levels reported by Soegiarto et al. (1981) for the Sunda Strait between Java and Sumatera, and probably reflect the nearness of our site to land and nutrient-rich, run-off water. Larval fish in the surface samples were much scarcer than anticipated, however, and were far fewer than found in the Sunda Strait study. The annual mean density was only 0.13/m^3, with a peak single-sample density of 1.80/m^3 at Bulak in 5 m of water in December. Clupeiform larvae contributed only 0.04/m^3 to the annual mean density.

The vertical sampling provided a very different set of data (Fig. 3). The average numbers (9 samples/month) of eggs were three times greater than in the horizontal hauls (1.89/m^3), with a peak density of 20.25/m^3 in 20 m of water in February. *Stolephorus* eggs contributed a density of 0.18/m^3 to the annual figure, with peaks of abundance in April and August. The mean density for larvae was 10 times greater than in the horizontal samples, with 1.26/m^3, and a peak value of 31.50/m^3 was obtained in 10 m of water in November. Clupeiform larvae contributed 30.00/m^3 to this single station peak value and 0.52/m^3 to the annual mean density, while their November average density was 4.00/m^3.

The densities data (Fig. 4) showed that fish eggs in the sampling area were most abundant in depths of 10-20 m, while both total clupeiform larvae were most abundant (or more easily caught) at 10 m.

On the basis of the annual means from the vertical hauls it was calculated that at least 7 x 10^9 eggs and 5 x 10^9 larvae were present at any time in the sampling area.

Fish eggs are the only items in the zooplankton which are fully at the mercy of tides and currents. Knowing that most of the pelagic eggs in the Java Sea are spawned at night and hatch within 24 hours (Delsman 1972), and knowing the surface current strengths and directions, it is possible to identify egg laying sites with a considerable degree of accuracy. For example, eggs which were abundant at the Mlonggo transect over 20 m of water in September would have been spawned there, as the drogue results showed no discernible current in the surface waters at this time, whereas the high densities of eggs found at Bulak in March were current affected and probably originated very close to Panjang Island, Jepara, the previous night.

Movements of eggs taken from the water column are less certain, as only surface currents were measured on a regular basis, and assessment of larval movements are more difficult still, as the larvae certainly undertake vertical migrations. However, one particularly interesting set of samples taken from 10 to 20 m of water at Bulak in November contained large numbers of clupeiform larvae which were estimated to be one week old. An attractive hypothesis was that these had been laid at the mouth of the River Wulan (a large perennial river with a catchment area of 2,000 km^2, joining the sea 10 km south of Bulak) and had then moved slowly northwards under the occasional influence of a 0.1-0.5 km/hr current to arrive in the sampling area as week-old larvae.

This study was helpful primarily in fish biology and systematics, while also developing the core of knowledge available in the region in terms of identification of the eggs and larvae of commercially important species. It has also had some value as a baseline study, and in developing a better understanding of current systems and their likely effects on inshore fish populations. Although the area covered was small, the program can be viewed as a pilot study, and the data could certainly be valuable in planning further larger surveys for resource management purposes.

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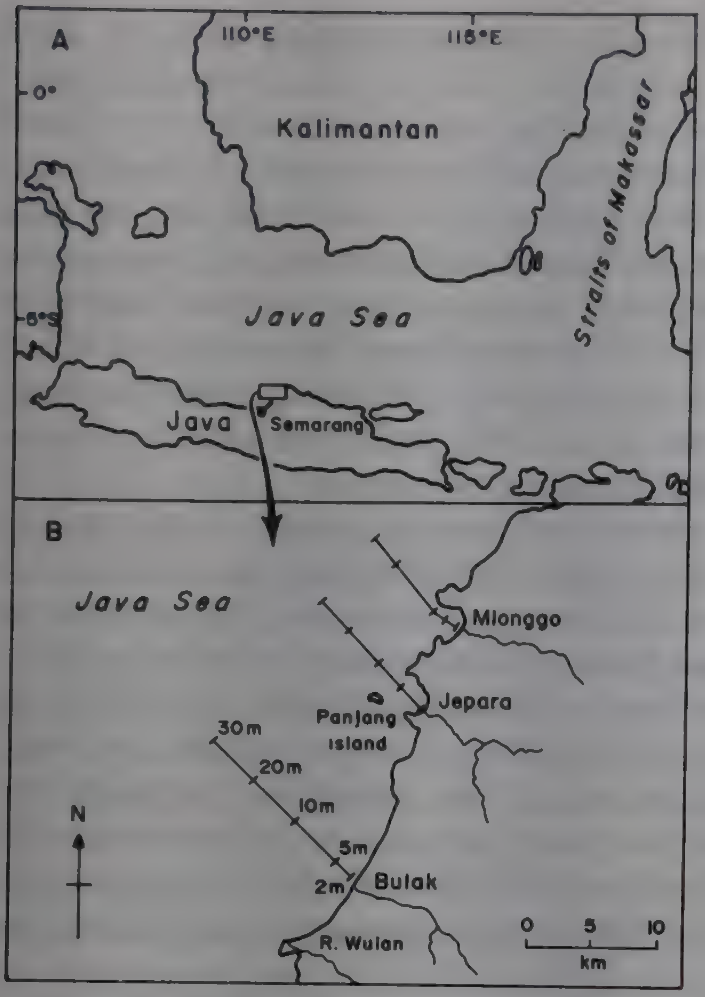
A
110°E
115°E
0°
5°S
Kalimantan
Straits of Makassar
Java Sea
Java
Semarang
B
Java Sea
Mlonggo
Jepara
Panjang island
30m
20m
10m
5m
2m
Bulak
R. Wulan
N
0 5 10
km

Fig. 1. A, Java Sea; B, sampling sites.

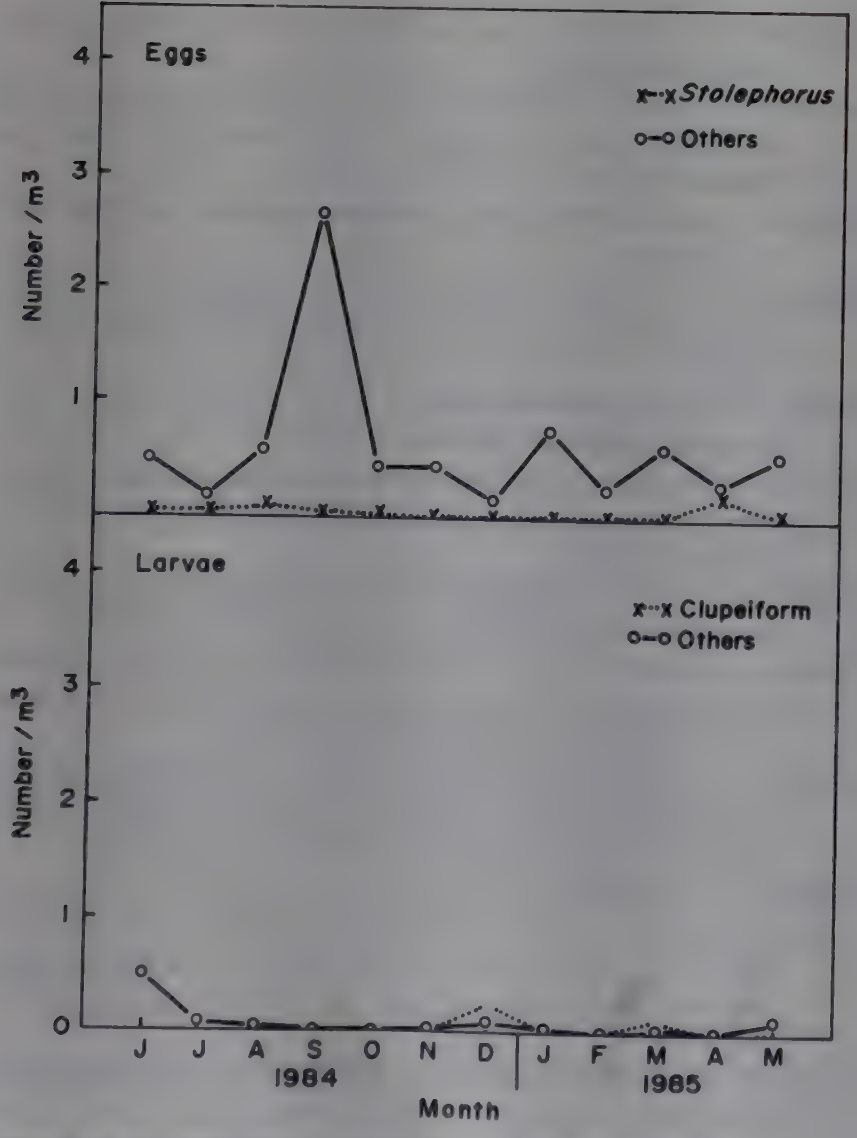

Fig. 2. Seasonal variation in numbers of fish eggs and larvae/m^3 from horizontal surface samples (15/month).

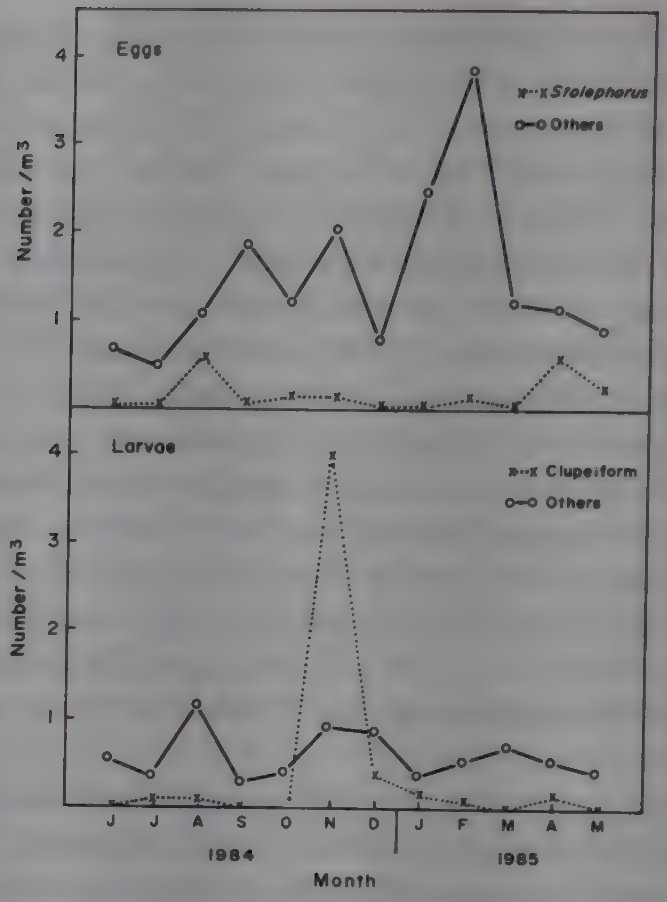

Fig. 3. Seasonal variation in mean numbers of fish eggs and larvae/m^3 from vertical samples (9/month).

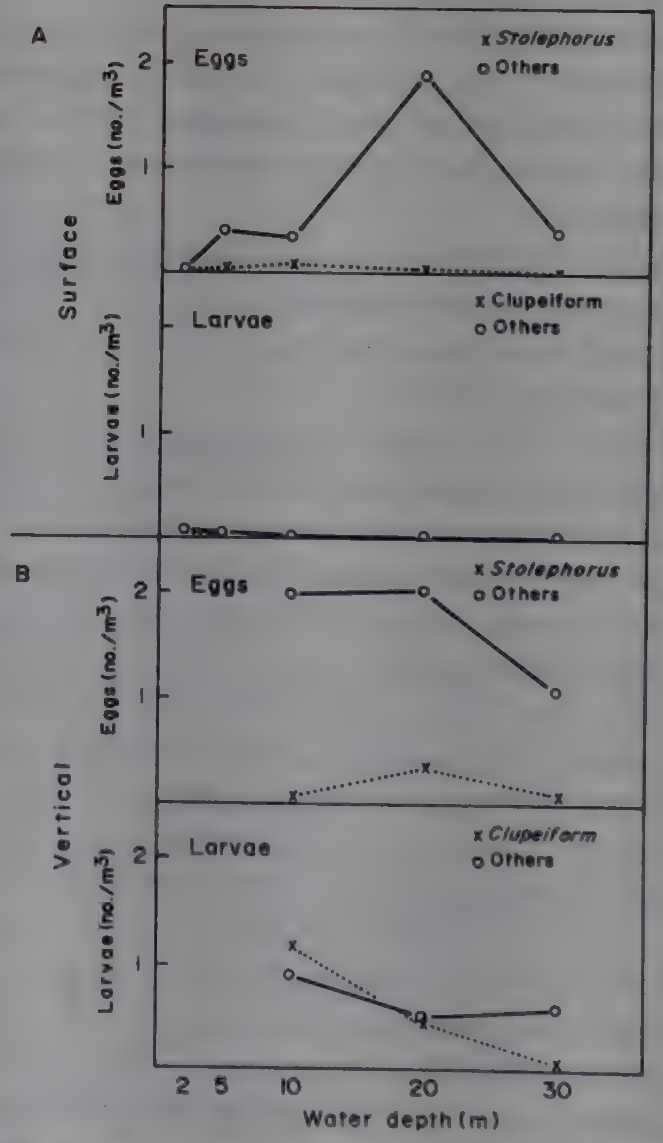

Fig. 4. Mean numbers of fish eggs and larvae/m^3 in or over water of different depths. A. Surface samples over water 2-30 m deep. B. Vertical samples through water 10-30 m deep.

Characteristics of Gliding Bacteria Isolated from Diseased Flounder, *Paralichthys olivaceus*

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Abstract

The morphological, biochemical and physiological characteristics of 23 strains of gliding bacteria isolated from diseased cultured flounder *(Paralichthys olivaceus)* in Japan in 1983 and 1985 were determined. The strains were classified into two groups: Group I (83%) was closely related to *Flexibacter marinus* and Group II (17%) was tentatively identified as another species of *Flexibacter* based on differences in biochemical and physiological characteristics.

Introduction

Gliding bacteria as disease agents of cultured freshwater and marine fish are well documented in Japan. *Flexibacter columnaris* has been isolated from diseased loach, *Misgurnus anguillicaudatus* (Wakabayashi and Egusa 1966); eel, *Anguilla japonica* (Wakabayashi et al. 1970); rainbow trout, *Salmo gairdneri;* and carp, *Cyprinus carpio* (Hatai and Hoshina 1971). Kimura et al. (1978) reported the isolation of a filamentous bacteria from gill lesions of two species of salmonids, *Salmo gairdneri* and *Oncorynchus masou*, while mortalities in yellowtail fry, *Seriola quinqueradiata*, have been reported to be caused by *Cytophaga* sp. (Kusada and Kimura 1982). Mass mortalities of cultured juvenile red sea bream, *Pagrus major*, and black sea bream, *Acanthopagrus schlegeli*, have been ascribed to a *Flexibacter* sp., where Hikida et al. (1979) proposed a new species, *Flexibacter marinus*, on the basis of biochemical tests and an obligate requirement of seawater for growth.

F. columnaris is normally regarded as a freshwater microorganism and its apparent appearance in a marine environment is unusual; however, it has been implicated as the causal agent of black patch necrosis in Dover sole, *Solea solea*, at Ayshire, Scotland (Campbell and Buswell 1982). A history of gliding bacteria infection in a closely-related fish species, the flounder *(Paralichthys olivaceus)*, has not been reported in Japan. This study investigated the morphological, biochemical and physiological characteristics of 23 strains of gliding bacteria isolated from diseased flounder.

Materials and Methods

The sources of 23 strains of gliding bacteria are summarized in Table 1. Diseased fish showed pale red discoloration of the skin and eroded ventral and caudal fins (Fig. 1); dermal lesions were observed in more advanced cases.

Bacteria were grown on *Cytophaga* agar containing 100% seawater. This medium is composed of tryptone (Difco), 0.05%; yeast extract (Daigo), 0.05%; bonito fish extract (Wako), 0.02%; sodium acetate, 0.02%; and agar (Difco), 1.0%. Cultures used as inocula for the various tests were grown on *Cytophaga* slants for 2 days at 25°C.

The organisms were stained by Gram's method after incubation for 24 hours at 25°C in *Cytophaga* agar. Cell morphology, size and motility were determined by examination of wet mounts with a phase contrast microscope. Microcyst formation was tested using the method of Dworkin and Gibson (1964) and the ability to form fruiting body was determined by inoculating the microorganisms on sterile fish tissues submerged in tap water (Ordal 1946).

The methods described by Pacha (1968), Pacha and Porter (1968), and Lewin and Lounsbery (1969) were used for the biochemical tests; routine procedures were taken from the Manual of Microbiological Methods (Society of American Bacteriologists 1957).

Cytophaga broth was used for the physiological tests. The composition of this medium is the same as *Cytophaga* agar except that agar is omitted. The seawater requirement of the bacteria was determined at 25, 30, 50, 75 and 100% seawater. Growth was determined by observing visible turbidity after incubation for 5 days at 25°C. Sodium chloride requirement was determined at 0, 1, 3, 5 and 7%; and pH tolerance tested from pH 4 to 10. Cultures were incubated for 2 weeks at 25°C. The ability of the strains to grow at 5, 15, 25, 30 and 37°C was observed after 7 days of incubation. Stabs were made into *Cytophaga* agar deeps supplemented with 1.0% glucose and 0.1% or 0.2% each of potassium nitrate or sodium bicarbonate to test for anaerobic growth (Simon and White 1971). Growth on solid medium was determined using Nutrient Agar (Nissui), BCP Semisolid Agar Base (Eiken), CTA Agar (Nissui), Trypto-Soya Agar (Nissue), Brain Heart Infusion (BHI) Agar (Difco), MacConkey Agar (Nissui) and BTB Teepol Agar (Nissui).

Results

All strains were gram negative flexuous rods, exhibited gliding motility in wet mounts, while colonies showed spreading characteristics on agar medium. The width of the cells did not vary much (0.1 to 0.2 μm) but the length showed much variation (3 to 20 μm). In microcyst media, spherical cells were observed in two-week old cultures of two strains, HF and 2408, by using Pfeiffer staining. The vegetative cells of strain 2408 were all converted to spherical bodies (Fig. 2) after 2 weeks of incubation at 25°C. None of the isolates produced fruiting bodies.

The morphological and biochemical characteristics of the strains are listed in Table 2. All strains were catalase and oxidase positive, hydrolized casein and tributyrin with variable hydrolysis of gelatin and tyrosine. Xanthine, arginine and esculin were not hydrolyzed. Hide powder was decomposed but not starch, agar, cellulose and chitin. Acid was not produced in tartrate, citrate and mucate. Glucose was not utilized, neither were the 25 carbohydrates tested: adonitol, arabinose, cellobiose, dextrin, dulcitol, erythritol, esculin, galactose, glyceral, inositol, inulin, lactose, levulose, maltose, mannitol, melezitose, melibiose, raffinose, rhamnose, salicin, sorbitol, starch, sucrose, trehalose and xylose. The microorganisms reduced nitrate and produced ammonium but not gas, indole and acetoin. Hydrogen sulfide production was diverse. Methyl red, Voges-Proskauer and urease tests were negative; tween 80 was hydrolyzed but diverse in tween 20. Congo red was adsorbed, litmus milk reduction was diverse, methylene blue was not reduced and all bacteria tested were lyzed. From the differences in characteristics, the strains were classified tentatively into two groups. Group I, 2403, 2405, 2408, HF, NE, GBFI, 2, 3, 5, 6, 11, 13, 14, 15 16, 17, 19, 20 and 22, showed pale yellow colonies, produced spherical structures in two strains, hydrolyzed tween 20 and did not reduce litmus milk. The strains of Group II, GBF10, 12, 18 and 21, showed dark yellow colonies, did not produce spherical bodies, did not hydrolyze tween 20, were negative in DNase test and reduced litmus milk.

The strains showed common physiological characteristics as shown in Table 3; however, the strains of Group II tolerated growth at 25% seawater and 37°C but failed to grow at pH 5 and 10. All strains were not able to grow anaerobically at any test concentration of sodium chloride.

Discussion

The strains fit the definition of the genus *Flexibacter*, originally described by Soriano (1974) and Buchanan and Gibbons (1974) and *Flexibacter* sp. reported by Sawyer (1976), except that the latter grow in sodium chloride and seawater as well. Based on the results of biochemical and physiological characteristics and confirmed by the numerical value obtained from S-value analysis (Sokal and Michener 1958), the strains are classified into two groups, Group I (83%) and Group II (17%). The strains of Group I showed similar characteristics as *F. marinus* reported by Hikida et al. (1979) but differed slightly in the seawater requirement for growth. The strains reported by Hikida et al. (1979) tolerated growth at a lower salinity (30%) while the present strains of Group I grew at 50% seawater. This may be partly explained by the salinity and quality of the seawater used. The strain from black sea bream studied by Kusuda and Kimura (1982) is similar to Group I except in urease production and growth on different media. Additional biochemical and physiological characterization of the strains in Group II is being conducted to confirm their identity.

Microcysts have not been previously reported in non-fruiting fish pathogenic gliding bacteria, although Anderson and Conroy (1969) noted "round bodies" in cultures from the eroded mouth of rainbow trout raised in seawater. The authors observed the organism as long slender myxobacterium producing round bodies but it was distinct from the appearance of *Sporocytophaga* sp. The taxonomic position of the organism, however, was not reported. Dworkin and Voelz (1962) reported microcyst formation in *Myxococcus xanthus* which involved shortening and thickening of vegetative cells. In this study, no clear evidence of microcyst formation was obtained although spherical structures were observed in two strains after 2-4 weeks of incubation but there was no thickening and shortening of vegetative rods after 4-5 days. Pacha and Ordal (1970) observed spheroplast-like structures in old cultures of *F. columnaris* which are probably involution forms or degenerative stages of the organism. However, the presence or absence of microcysts is no longer regarded as a useful criterion for classification, as this property is largely dependent on cultural conditions. Moreover, the two isolates where spherical structures were observed were closer to *F. marinus* rather than *F. columnaris* and are tentatively classified as such until further characteristics are investigated to confirm their taxonomic position.

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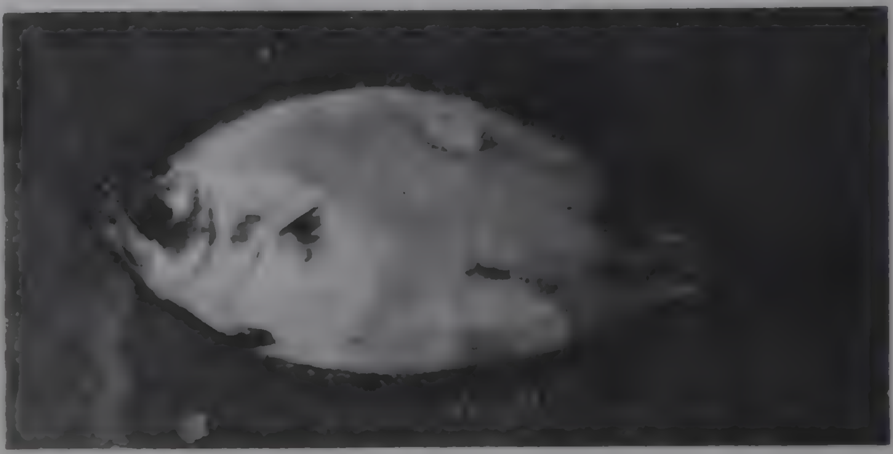

Fig. 1. Diseased flounder exhibiting pale red discoloration of the skin and eroded ventral and caudal fins.

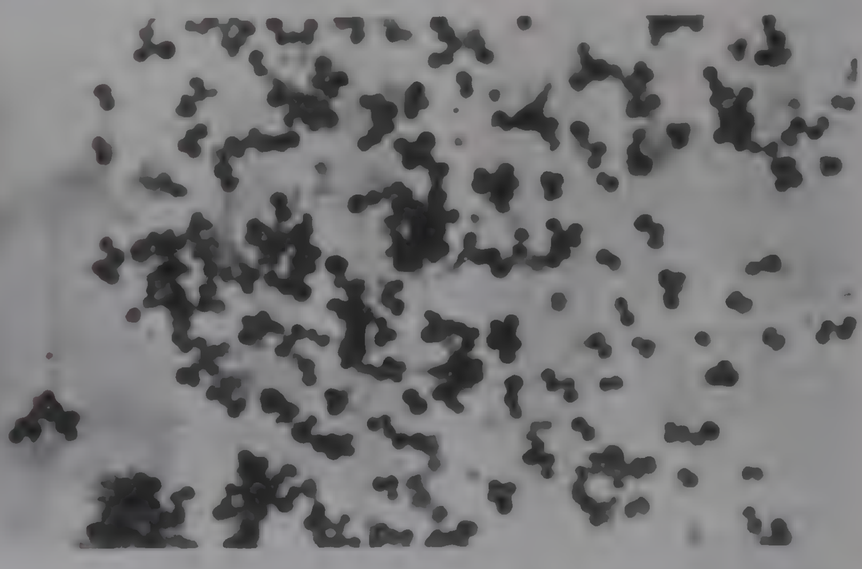

Fig. 2. Spherical structures produced by strain 2408 in microcyst medium after 2 wk of incubation at 25°C. Pfeiffer stain, 1,000 x.

Table 1. Sources of 23 strains of gliding bacteria from flounder used in morphological biochemical and physiological studies.

Strain	Source of isolation	Location and date
2403	body surface	Nagasaki, Feb. 1983
2405	eye surface	Nagasaki, May 1983
2408	body surface	Nagasaki, Dec. 1983
HF	body surface 5-10 g fry*	Ehime, May 1985
NE	body surface	Nagasaki, Apr. 1985
GBF1-6	body surface	Hiroshima, Aug. 1985
GBF10-22	body surface lesions 61-73 mm body length*	Hiroshima, Aug. 1985

*Entries with sizes were isolated from fry; the rest were from adult flounder.

Table 2. Morphological and biochemical characteristics of gliding bacteria isolated from flounder.

Characteristics	All isolates[1] (23 strains)	Group I[2] (19 strains)	Group II[3] (4 strains)
Gram	–		
Colony color	d	pale yellow	yellow
Motility	+		
Cell length (μm)	3 ~ 20	3 ~20	4 ~10
Spherical body	d	+(2/19)	–
Fruiting body	–		
Catalase	+		
Oxidase	+		
Hugh-Leifson test	–		
Starch	–		
Agar	–		
Cellulose	–		
Casein	+		
Gelatin	d	+(17/19)	+
Chitin	–		
Hide powder	+		
Tyrosine	d	3(11/19)	+
Tributyrin	+		
Xanthine	–		
Arginine	–		
Esculin	–		
Tartrate	–		
Citrate	–		
Mucate	–		
25 Carbohydrates	–		
Nitrate	+		
Hydrogen sulfide	d	+(3/19)	+(3/4)
Gas	–		
Indole	–		
Acetoin	–		
Ammonium	+		
Methyl red	–		
Voges-Proskauer	–		
Urease	–		
DNase	d	+	–
Lipase			
Tween 20	d	+	–
Tween 80	+		
Congo red	+	+	w
Litmus milk	d	–	+
Methylene blue	–		
Bacterial lysis			
Aeromonas hydrophila	+		
Edwardsiella tarda	+		
Vibrio anguillarum	+		
Escherichia coli	+		

a) Group I = 2403, 2405, 2408, HF, NE, GBF1, 2, 3, 5, 6, 11, 13, 14, 15, 16, 17, 19, 20, 22
b) Group II = GBF10, 12, 18, 21
c) d = diverse; ( ) = expressed as number of cultures positive/number of cultures tested
d) 25 carbohydrates = see text
e) w = weakly positive

[1] Common characteristics of Groups I and II.

[2] Characteristics of Group I which differ from Group II.

[3] Characteristics of Group II which differ from Group I.

Table 3. Physiological characteristics of gliding bacteria isolated from flounder.

Characteristics	All isolates (23 strains)	Group I (19 strains)	Group II (4 strains)
Growth on			
Nutrient Agar	d	+(14/19)	+
BCP Semisolid Agar Base	d	+(18/19)	+
CTA Agar	+		
TSA	d	+(6/19)	+
BHI Agar	d	–	+
MacConkey Agar	–		
BTB Teepol Agar	–		
Growth in			
Seawater (%)			
100	+		
75	+		
50	+		
30	d	–	+
25	d	–	+
Sodium Chloride (%)			
7	–		
5	–		
3	–		
1	–		
0	–		
pH			
10	d	+(10/19)	–
9	+		
8	+		
6	+		
5	d	+	–
4	–		
Temperature (°C)			
37	d	–	+
30	+		
25	+		
15	+		
5	–		

a) Group I = 2403, 2405, 2408, HF, NE, GBF1, 2, 3, 5, 6, 11, 13, 14, 15, 16, 17, 19, 20, 22
b) Group II = GBF10, 12, 18, 21
c) d = diverse; ( ) = expressed as number of cultures positive/number of cultures tested

The Pathogenicity of Bacteria Associated with Transport-Stressed *Chanos chanos* Fingerlings*

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Abstract

Two isolates of *Aeromonas hydrophila* biovar *hydrophila* and two isolates of *Pseudomonas*-like bacteria cultured from milkfish, *Chanos chanos*, fingerlings stocked in Laguna Lake (Philippines) pens after transport were tested for virulence against healthy milkfish fingerlings. Various combinations of bacterial concentration and different routes of inoculation (intraperitoneal injection, bath treatment of scaled fish and bath treatment of unscaled fish) were tested. Results show that bacterial entry in the pathogenesis of the test bacteria to challenged fish is more effective in fishes with scales removed than in uninjured fish or through intraperitoneal injection. The minimum lethal dose of *A. hydrophila* to scaled fish appeared less than 10^5 cells/ml of water medium. For *Pseudomonas*-like isolates, the minimum lethal dose was at the level of 10^5 cells/ml of water medium. Fish mortalities significantly increased on day 2 in all bacteria but *A. hydrophila* was significantly more virulent than the *Pseudomonas*-like inocula. When *A. hydrophila* was injected intraperitoneally into undamaged fish, the maximum dose of 10^7 cells per 2-g fish was needed to cause significant mortalities. In general, virulence of the isolates was directly proportional to dose as well as to the length of exposure. Acute signs of bacterial hemorrhagic septicemia were prominently manifested among experimentally infected scaled milkfish. Lethargic fish displayed spastic and unbalanced swimming movements before death. The virulence of the bacteria as evaluated in this study should lead to further investigations on the role of extracellular factors in bacterial pathogenesis to milkfish fingerlings.

Introduction

Commercial culture of milkfish, *Chanos chanos*, in fishpens at Laguna Lake, Philippines, significantly contributes to its overall production in the Philippines. However, heavy mortalities after stocking of fingerlings in the lake suggest bacterial infection not experimentally demonstrated (Duncan 1974; Lio-Po 1984). A recent study on milkfish fingerlings transported in "petuya" boats from brackishwater ponds to the lake fishpens identified adverse environmental and stress conditions, including bacteria associated with the seeded fish, namely, *Aeromonas hydrophila* biovar *hydrophila* and two isolates resembling *Pseudomonas* (Lio-Po et al., this vol.).

Aeromonas hydrophila and *Pseudomonas* spp. had been reported as causative agents of bacterial hemorrhagic septicemia (Meyer and Collar 1964; Hatai et al. 1975; Jo and Ohnishi 1980; Tonguthai 1985; Wang Xu 1985). Fishes were generally predisposed to these opportunistic pathogens by adverse environmental conditions as well as by handling and crowding stress. Among milkfish, no study on the virulence of these bacteria has been reported. Hence, this study aimed to determine the pathogenicity of these bacterial isolates to healthy milkfish fingerlings and to identify the most efficient route of bacterial entry into the fish.

Materials and Methods

Pure cultures of four bacterial isolates recovered from transport-stressed *Chanos chanos* fingerlings were selected for challenge experiments with healthy milkfish (Lio-Po et al., this vol.). The bacterial isolates, #116 and #128, were earlier identified as *Aeromonas hydrophila* biovar *hydrophila* while isolates #57 and #160 were preliminarily classified as *Pseudomonas* spp. These microorganisms were maintained in nutrient agar and mass-produced in trypticase soy agar at temperatures of 28-30^{o}C after 24-hour incubation. The fish, 1.4-2.4 g, were obtained from brackishwater ponds in Iloilo and gradually acclimated to freshwater conditions with ultraviolet sterilized water.

Five fish were stocked in each 10-l glass aquarium filled up to 5 l and provided with adequate filtered aeration. Three routes of pathogen inoculations were tested: (1) bacterial bath of scaled fish; (2) bacterial bath of unscaled fish; and (3) intraperitoneal injection (IP). Scaling of fish consisted of aseptic scraping off of scales over a 1-cm^2 area of the left epaxial skin with the blunt side of a sterile scalpel. For bacterial bath treatments, five doses were tested at tenfold increasing dilutions/ml of rearing water medium, including controls. Intraperitoneal injection experiments similarly involved tenfold increasing dilutions in six doses. Feed was withheld two days prior to and throughout the experiments. Three trials for bath treatment of scaled and unscaled fish and two trials of intraperitoneal injections were conducted. Bacterial reisolations were made from lesions and kidneys of exposed and control fish. Recovered bacteria were presumptively identified to confirm their resemblance to the original inoculum.

The results obtained were analyzed by the analysis of variance and Duncan's multiple range test at 1% and 5% levels of significance. The median lethal doses (LD_{50}) were estimated by using the graphical process with probit/log dose transformation and adjustment for natural response was computed using Abbott's formula (Roberts and Boyce 1972).

Results

Milkfish exposed to the four bacterial isolates exhibited signs of disease and mortalities. Usually scaled fish manifested external signs of abdominal swelling, skin blanching and hemorrhagic fin bases, skin, head or periorbital area. Internally, fish variably exhibited hyperemic viscera and pale kidneys. Septicemia was likewise evident from the positive isolations from kidney and lesions which resembled the initial inocula. Lethargy and spastic and unbalanced swimming before death were also observed. Among unscaled fish, abdominal distension was common. Mortality patterns are summarized in Table 1 for scaled fish, Table 2 for unscaled fish and Table 3 for intraperitoneally-injected fish.

Infection experiments on scaled fish showed no significant differences ($P < 0.01$) between isolates #116 and #128. Disease effects were apparent at exposures to the lowest dose of 2.70 and 4.35 x 10^5 cells/ml, respectively, although maximum mortalities were evident at the x 10^6 level of bacterial inoculum. Deaths were observed as early as 12 hours after exposure and gradually increased daily until the second day. Isolates #57 and #160 similarly did not significantly differ from each other in terms of virulence ($P < 0.01$). In both isolates, exposure to 7.5 x 10^4 and 6.5 x 10^4 doses, respectively, was not fatal. Some mortalities, however, were observed among #57-inoculated fish on day seven after exposure. Increasing the dose to the 10^5 and 10^6 levels proved lethal. Maximum mortalities at these doses occurred on days 1-3 after exposure.

Among fish without dermal abrasion, no significant differences among bacterial strain inocula were observed ($P > 0.05$). Isolates #116, #128, #57 and #160 produced maximum mortalities at the highest dose given on days 0.5, day 1, day 2 and day 5, respectively. The first three inocula, likewise, did not yield significant deaths in lower doses even at longer exposure ($P < 0.01$). In isolate #160, however, at 6.5 x 10^5 dose after six days, all fish died.

Fish injected intraperitoneally with bacteria significantly differed in their responses to the different inocula. With both #116 and #128 and a dose of 5.97 and 8.7 x 10^7, respectively, almost immediate death of all resulted. Lower doses, however, yielded sporadic deaths throughout the 10-day period. Isolates #57 and #160 similarly yielded erratic levels of mortalities at varying test doses.

Estimates of the median lethal doses were determined for data obtained 12 hours after bath exposure of scaled milkfish (Table 4). Results indicate no relative difference in virulence after 12-hour exposure. The LD_{50} for unscaled fish similarly exposed to the test bacteria was not possible. For intraperitoneally-injected fish, LD_{50} values at day 10 are presented in Table 5. Isolate #57 showed lower LD_{50} than isolates #128 and #160.

Discussion

Koch's requisites for confirming the bacterial isolates' pathogenicity to healthy milkfish fingerlings were clearly complied with in these experiments. Manifestations of disease were observed and the inoculated bacteria were recovered from the test fish. Pathology appeared pronounced among scaled fish compared to uninjured fish or intraperitoneal injection. After initial exposure to *A. hydrophila*, a rapid onset and severe pathogenesis led to mortalities in less than 12 hours even at the lowest dose of 10^5 cells/ml water medium. Cipriano et al. (1984) mentioned that acute effects can be exhibited by *A. hydrophila* on susceptible fishes within a few hours following exposure. Signs of bacterial hemorrhagic septicemia were, in fact, demonstrated among challenged milkfish, notably hemorrhagic lesions and lesion blanching, particularly, on injured sites where scales were removed. Such were similarly observed among naturally infected milkfish (Lio-Po et al., this vol.). Furthermore, septicemia was apparent from the heavy growth of pure bacterial isolates recovered from the kidney of weak or freshly-dead fish.

Maximum mortalities were seen two days after initial exposure to bacteria. Hence, although the LD_{50} was at the level of 10^6 in 12 hours, in two days a lower LD_{50} level can be postulated. Newman (1983) cited studies with other fishes showing that 10^3 cells of *A. hydrophila* plus handling and scale removal caused *Aeromonas* infection. Huizinaga et al. (1979) used 10^6 cells/ml water medium to induce disease experimentally among largemouth bass. In addition, Kaper et al. (1981) reported that in Chesapeake Bay waters, *A. hydrophila* occurred normally at levels of 10^3 cells/ml, increasing when eutrophic conditions occur. Assuming similar conditions in Laguna Lake waters, stocking milkfish fingerlings with scales removed from handling and crowding during transport may lead to subsequent infection. Bejerano (1984) showed that stress can increase susceptibility of *Sarotherodon aureus* to *A. hydrophila*. Moreover, scale removal enhanced *Aeromonas* infections among golden shiners (Lewis and Bender 1961).

The *Pseudomonas*–like bacteria exhibited similar pathology to *A. hydrophila* but resulting deaths were limited to levels of 10^5 cells/ml rearing medium. This agrees with reports of Lewis and Plumb (1979) and Bullock and McLaughlin (1970) on parallelism in pathology by both organisms to susceptible fishes. It is also interesting to note that these organisms are ubiquitous in water (Evelyn and McDermott 1961; Horsely 1977). In fact, they show better survival in water than the obligate pathogens *Aeromonas salmonicida* and *Chondrococcus columnaris* (Ross and Smith 1974). That both organisms are opportunistic pathogens is confirmed by this study which show that unscaled fish remain unaffected at the 10^5 cells/ml dose. Higher bacterial concentrations of 10^8 and 10^6 for *A. hydrophila* and *Pseudomonas*-like inocula, respectively, were required to effect significant mortalities.

Several previous studies have implicated extracellular enzymes in the pathogenesis of *A. hydrophila* and *Pseudomonas*. Similarly, in the pathogens of milkfish fingerlings confirmed in this study, the role of these extracellular proteins need to be investigated.

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*SEAFDEC Contribution No. 201

Table 1. Cumulative mortality rates (%) of scaled *Chanos chanos* fingerlings challenged with test bacteria by immersion at five fish/treatment.

Isolate no	Dose per ml	Day 0.5	1	P.226 2	3	4	5	6	7	8	9	10
116	0	0	–	20	–	–	–	–	–	–	40	–
	4.35×10^5	80	–	–	–	–	–	–	–	–	–	–
	$\times 10^6$	80	–	100	–	–	–	–	–	–	–	–
	$\times 10^7$	80	–	100	–	–	–	–	–	–	–	–
	$\times 10^8$	100	–	–	–	–	–	–	–	–	–	–
128	0	0	–	20	–	–	–	–	–	–	40	–
	2.70×10^5	40	80	100	–	–	–	–	–	–	–	–
	$\times 10^6$	40	100	–	–	–	–	–	–	–	–	–
	$\times 10^7$	80	100	–	–	–	–	–	–	–	–	–
	$\times 10^8$	100	–	–	–	–	–	–	–	–	–	–
57	0	0	–	20	–	–	–	–	–	–	40	–
	7.50×10^3	0	20	–	–	–	–	–	–	–	–	–
	$\times 10^4$	0	–	20	–	–	–	–	40	–	80	–
	$\times 10^5$	20	60	80	100	–	–	–	–	–	–	–
	$\times 10^6$	80	–	100	–	–	–	–	–	–	–	–
160	0	0	–	20	–	–	–	–	–	–	40	–
	6.50×10^3	0	40	–	–	–	60	–	–	–	–	–
	$\times 10^4$	0	–	–	–	–	–	–	–	20	40	–
	$\times 10^5$	20	80	100	–	–	–	–	–	–	–	–
	$\times 10^6$	80	100	–	–	–	–	–	–	–	–	–

– = same as preceding day.

Table 2. Cumulative mortality rates (%) of unscaled *Chanos chanos* fingerlings challenged with test bacteria by immersion at five fish/treatment.

Isolate no.	Dose per ml	Day 0.5	1	2	3	4	5	6	7	8	9	10
116	0	0	–	–	–	–	–	–	–	–	–	–
	4.35×10^5	0	–	–	–	–	–	20	–	–	–	–
	$\times 10^6$	0	–	–	–	–	–	–	–	–	–	–
	$\times 10^7$	0	–	–	–	–	20	–	40	–	–	–
	$\times 10^8$	100	–	–	–	–	–	–	–	–	–	–
128	0	0	–	–	–	–	–	–	–	–	–	–
	2.70×10^5	0	–	–	–	–	–	–	–	–	–	–
	$\times 10^6$	0	–	20	–	–	–	–	–	–	–	–
	$\times 10^7$	0	–	–	–	–	–	20	–	–	–	–
	$\times 10^8$	40	100	–	–	–	–	–	–	–	–	–
57	0	0	–	–	–	–	–	–	–	–	–	–
	7.50×10^3	0	–	–	–	–	–	–	–	–	–	40
	$\times 10^4$	0	–	–	–	–	–	–	–	–	–	–
	$\times 10^5$	0	–	–	–	–	–	20	–	–	–	40
	$\times 10^6$	–	–	100	–	–	–	–	–	–	–	–
160	0	0	–	–	–	–	–	–	–	–	–	–
	6.50×10^3	0	–	–	–	–	–	–	–	–	–	–
	$\times 10^4$	0	–	–	–	–	–	20	–	40	–	–
	$\times 10^5$	0	–	–	–	–	40	100	–	–	–	–
	$\times 10^6$	20	–	–	60	80	100	–	–	–	–	–

– = same as preceding day.

Table 3. Cumulative mortality rates (%) of *Chanos chanos* given intraperitoneal injection of test bacteria at five fish/treatment.

Isolate no.	Dose per fish	Day 0.5	1	2	3	4	5	6	7	8	9	10
116	0	0	–	–	–	–	–	–	–	–	–	–
	8.70×10^3	0	0	0	0	20	–	–	–	–	–	–
	$\times 10^4$	0	0	0	0	0	20	–	–	–	–	40
	$\times 10^5$	0	–	–	–	–	–	–	–	–	–	–
	$\times 10^6$	40	–	–	–	–	–	–	–	–	–	–
	$\times 10^7$	100	–	–	–	–	–	–	–	–	–	–
128	0	0	–	–	–	–	–	–	–	–	–	–
	5.40×10^3	0	–	–	–	–	–	–	–	–	–	–
	$\times 10^4$	0	–	–	–	–	20	–	–	40	–	–
	$\times 10^5$	0	20	–	–	–	–	–	40	–	–	–
	$\times 10^6$	40	60	–	–	–	–	–	–	–	–	–
	$\times 10^7$	100	–	–	–	–	–	–	–	–	–	–
57	0	0	–	–	–	–	–	–	–	–	–	–
	1.50×10^3	0	–	–	–	–	–	–	20	–	–	–
	$\times 10^3$	0	–	–	20	–	–	–	–	40	–	–
	$\times 10^4$	0	–	–	–	–	–	–	–	–	–	20
	$\times 10^5$	0	–	–	–	–	–	20	60	–	80	–
	$\times 10^6$	60	–	–	–	–	–	–	–	–	–	–
160	0	0	–	–	–	–	–	–	–	–	–	–
	1.30×10^3	0	–	–	–	–	–	–	–	–	–	20
	$\times 10^3$	0	–	–	–	–	–	–	–	–	–	–
	$\times 10^4$	0	–	–	–	–	–	–	–	20	–	–
	$\times 10^5$	0	–	–	–	–	–	–	20	40	–	60
	$\times 10^6$	0	–	–	–	–	–	–	–	–	–	20

– = same as preceding day.

Table 4. Approximate median lethal dose (cells/ml) after 12-hour exposure by bath of scaled milkfish fingerlings to test bacteria.

Isolate no.	LD_{50}	95% confidence limits	Variance	χ^2
128	1.16×10^6	1.43×10^5 to 9.40×10^6	0.22	1.48^{ns}
57	2.35×10^6	6.60×10^5 to 8.70×10^6	0.08	1.53^{ns}
160	2.04×10^6	5.55×10^5 to 7.54×10^6	0.08	1.53^{ns}

ns = no significant heterogeneity means that the fitted line in the graphical estimation of the LD_{50} is valid.

Table 5. Approximate median lethal dose (cells/2 g fish) at day 10 of milkfish fingerlings injected intraperitoneally with test bacteria.

Isolate no.	LD_{50}	95% confidence limits	Variance	χ^2
128	4.37×10^5	5.45×10^4 to 3.56×10^6	0.22	2.63^{ns}
57	3.63×10^4	4.35×10^3 to 3.04×10^5	0.27	4.63^{ns}
160	2.54×10^5	7.64×10^3 to 8.52×10^6	0.61	5.70^{ns}

ns = no significant heterogeneity means that the fitted line in the graphical estimation of the LD_{50} is valid.

Disease Investigation of Transported *Chanos chanos* Stocked in Laguna Lake, Philippines*

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Abstract

Milkfish, *Chanos chanos* fingerlings transported by boat *(petuya)* for seven to eight hours were observed for stress-inducing factors during transport, and daily for 10 days after stocking in pens in Laguna Lake, Philippines. Handling, hauling, and crowding of fish contributed to stressful conditions. Likewise, the transport procedure of closing the water entry hole at the bottom of the boat to block water exchange was associated with decreased dissolved oxygen and increased turbidity, with respective values of 2.4 mg/l and 79 Formalin Turbidity Units when water change was possible. In addition, marked and abrupt fluctuations in salinity from 15 to 30 ppt then 0 ppt within 1 to 3 hr were observed during transport. Although mortalities during transport were minimal, subsequent deaths after stocking in pens mounted to not less than 2.4%. Reddish snout, scale loss and hemorrhagic areas along the lateral body surface were observed. Bacterial counts of water increased significantly during transport when water exchange was stopped. Kidney and skin/muscle specimens yielded significantly higher bacterial counts on days 2, 3, 5 and 8 poststocking predominated by *Aeromonas hydrophila* biovar *hydrophila* and two species of gram-negative bacilli phenotypically resembling *Pseudomonas* sp.

Introduction

Newly caught wild or pond-reared milkfish fingerlings can be transferred directly to freshwater or vice versa without any ill effects. However, if these fingerlings undergo transport before direct transfer to freshwater, mass mortality may occur a few hours or days after stocking, even in environments with optimal conditions (Duncan 1974; Villaluz 1984). These mortalities within three days of stocking after transport can run as high as 35% (Smith 1978). Unconfirmed bacterial infection has been suspected as the eventual cause of death (Lio-Po 1984). This report details the physical, environmental and bacterial changes during transport of milkfish fingerlings by boat from brackishwater ponds to a freshwater lake and identifies the bacterial pathogens associated with subsequent mortalities.

Materials and Methods

The routine procedure of transporting milkfish fingerlings by *petuya* (boat) from brackishwater ponds at Taliptip, Bulacan, to freshwater fishpens in Laguna Lake, Philippines, was conducted. Milkfish, *Chanos chanos*, fingerlings of 0.73-4.90 g were collected the day before into a catching pond. Harvesting was done by seining, manually loading the fish into the *petuya* in 20- to 30-l tin cans. Each *petuya* contained from 47,500 to 187,000 fish per trip.

The *petuya* had a wooden, rectangular fish holding compartment of approximately 9 x 3.5 x 1 m or a stock density of no less than 5,000 fish/m^3. Water entered this compartment through a floor hole, about 8 to 10 cm in diameter, covered with a screen then was simultaneously pumped out for water exchange during the transport period. However, when passing through polluted waters along Manila Bay and in the Pasig River, the floor hole was closed for approximately 3 hours. Water then was recirculated. Upon reaching the nursery fishpens in Laguna Lake, the fish were unloaded and seeded into the fishpens also using the tin cans. The transport period lasted for 7 to 8 hours.

Water and fish specimens were collected throughout the operation. A sufficient number of transported fish were stocked separately in a *hapa* net for daily sampling for 10 days after seeding. Fish conditions, behavior and mortality data were recorded. Determinations of salinity, pH, temperature, dissolved oxygen, turbidity, hydrogen sulfide and ammonia were made using a refractometer and the DREL/5 Hach kit.

Bacterial counts in colony forming units (CFU) per ml of water or per cm^3 of fish tissue were conducted on trypticase soy agar (TSA). Identification was done applying standard identification procedures. Cultures were incubated at 25-30°C for 48 hours.

The analysis of variance was employed in the statistical evaluation of data at 0.01 and 0.05 levels of significance. Comparison of means was based on Duncan's multiple range test.

Results

For *petuya*-transported milkfish, stressful conditions were apparent from the time these were collected and crowded into the catching pond, handled during seining, hauling, counting and stocking in the boat, and crowded in the petuya while being transported. Measurements of the physicochemical parameters (Fig. 1) indicated abrupt fluctuations in salinity, dissolved oxygen and turbidity with high levels of ammonia during the transport period. The drastic variation in salinity from an initial 15 ppt before transport to 30 ppt during transport while passing through Manila Bay to 0 ppt upon reaching the lake were highly significant ($P < 0.01$). Dissolved oxygen was significantly lower ($P < 0.05$) at 2.4 mg/l in the catching pond as well as during transport (closed) than in the fishpen (4.2 to 10.4 mg/l). Ammonia levels, on the other hand, were significantly higher ($P < 0.01$) at 2 mg/l from the source and throughout the duration of the transport period relative to pen levels of 0.13 to 0.52 mg/l. Turbidity change from 47 FTU (Formalin Turbidity Units) before transport to 8 FTU during transport (open) to 79 FTU (closed) were not significantly different from each other. Furthermore, pH and temperature gave no significant variation, while hydrogen sulfide was consistently nil.

Mortalities observed during transport and upon arrival at the fishpen area did not exceed a total of ten fish. Subsequent daily mortalities in the pens within a 10-day period ranged from 1,000 to 28,000 fish. Cumulative mortality was estimated as 2.4%, a conservative estimate as only floating dead fish were enumerated. Among surviving fish after transport, manifestations of reddish snout, scale loss, fin and skin damage, and monolateral blindness were common. In addition, after seeding in pens, the fish developed lateral skin hemorrhagic spots and lesions, caudal fin rot and hemorrhagic fin bases.

Bacterial load of water ranged from 1×10^3 to 2.12×10^6 CFU/ml (Fig. 2). Analysis of variance indicated significantly higher counts of 10^6 levels ($P < 0.01$) during transport (closed). Among fish eye samples, a significant increase was seen on days 3, 7 and 8 after seeding. More frequently, significantly elevated counts of 10^6 were detected from kidney sections on days 2, 3, 5, 8 and 10 after seeding. Skin/muscle specimens, similarly, yielded increased counts (10^5) on days 2, 3, 5, 8 and 9 after seeding. Fish specimens before and during transport did not demonstrate significantly high bacterial levels.

A total of 161 bacterial isolates were recovered which predominantly consisted of gram-negative rods represented by isolates #57, #116, #128 and #160. Isolates #116 and #128 showed the highest prevalence and were identified as *Aeromonas hydrophila* biovar *hydrophila* (Schubert 1974; Popoff and Veron 1976) while isolates #57 and #160 cannot be specifically classified with any known bacteria but seem related to *Pseudomonas* sp. (Table 1).

Discussion

The crowded condition of the fish in the catching pond after harvest and when stocked in the boat for transport most likely caused physical damage to the fish. Lewis and Bender (1960) reported similar skin damage on golden shiners after harvest which paved the way for development of infections by *A. hydrophila*. Among mackerels *(Scomber scombrus* L.*)*, experimental demonstration of crowding effects, wherein 1,000 fish/m^3 crowded for 15 to 45 min., resulted in 40-100% mortalities after stocking in larger rearing systems (Lockwood 1981). Also, *Oreochromis aureus*, when experimentally subjected to stress from either handling, thermal shock, or social confrontations became three times more sensitive to *A. hydrophila* infections causing fish losses of 10-20% (Bejerano 1984). Adverse environmental parameters such as abrupt salinity fluctuation for milkfish is a particularly critical factor, as severe mortality after transport stress has been shown (Villaluz et al. 1982). In addition, fluctuating and low dissolved oxygen levels are stressful. Similar conditions have been implicated as predisposing fish to *A. hydrophila* infections (Haley et al. 1967; Meyer 1978; Walters and Plumb 1980). Likewise, since ammonia levels were significantly higher during transport vis-a-vis in pens compounded by low oxygen concentration, an enhancement of this toxicant's effect on exposed fish may be expected (Smart 1981).

Meanwhile, the significant bacterial increase in the transport water (closed) was possibly due to metabolic accumulation in the absence of water exchange. This may further explain the higher levels of turbidity at that time. In kidney and skin/muscle tissues, an almost simultaneously correlated increase to significant levels was noted after seeding, implying a close link in the development of pathological changes in these organs. Significant levels were initially noted in both organs on day 2 after stocking which continued, although alternately most times, until the last sampling period. That *A. hydrophila* was most frequently recovered from the milkfish samples after seeding in the lake strengthens the contention that transport stress conditions promote the development of subsequent bacterial infection.

Hence, milkfish mortalities during transport were acute effects of stress while those occurring after seeding in pens resulted from subsequent infections with *A. hydrophila* or *Pseudomonas*-like organisms. These species are ubiquitous in water, mud, healthy fish and other freshwater organisms (Lewis and Bender 1961; Evelyn and McDermott 1961; Horsely 1977; Ellis 1982; Nieto et al. 1984). Furthermore, the occurrence of fish infections due to *A. hydrophila* in eutrophic lakes has been earlier reported (Shotts et al. 1972). Thus, the difficult task of economically minimizing stressful conditions during transport or preventing subsequent bacterial infections remains to be explored.

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Table 1. Characteristics of bacterial isolates 116, 128, 57 and 160.

Characteristic	Isolate no. 116	128	57	160
Growth in NA	+	+	–	–
Growth in NB at 37°C	+	+	nt	nt
Growth at 7.5% NaCl	–	–	nt	nt
Growth in McConkey agar	+	+	–	–
Brown pigment	–	–	nt	nt
Amino acids as sole source of carbon:				
L-arginine	+	+	+	+
L-alanine	+	+	+	+
L-asparagine	+	+	+	+
L-histidine	+	+	–	–
L-serine	+	+	+	+
Butanediol dehydrogenase	+	+	–	–
Lipase	+	+	–	–
Lecithinase	+	+	–	–
Casein	+	+	+	+
Phosphatase	+	+	–	+
DNase	+	+	–	–
Cytochrome oxidase	+	+	+	–
Gluconate oxidase	–	–	–	–
Gelatin liquefaction	+	+	+	+
Urease	–	–	–	–
Peptonization of litmus milk	+	+	+	+
Starch hydrolysis	+	+	–	–
Malonate	–	–	–	–
Phenylalanine	–	–	–	–
Production of:				
Gas from glycerol	+	+	–	–
Gas from glucose	+	+	–	–
TSI reaction:				
Slant/butt	K/A	K/A	A/K	A/K
Gas	+	+	–	–
H_2S	–	–	–	–
SIM:				
H_2S	sl+	sl+	–	–
Motility	+	+	–	–
Simmon's citrate	+	+	–	+
Indole in tryptophan	+	+	–	–
Methyl red	–	sl+	–	–
Voges Proskauer	+	+	–	–
Decarboxylase:				
Arginine	+	+	+	–
Lysine	+	+	–	–
Ornithine	–	–	–	–
Acid from:				
Arabinose	–	–	–	–
Adonitol	–	–	–	–
Cellobiose	–	–	–	–
Dextrin	A	A	–	–
Erythritol	–	–	–	–
Esculin	–	–	–	–
Fructose	A	A	–	–
Dulcitol	–	–	–	–
Glucose	A	A	A	A
Glycerol	A	A	–	–
Galactose	A	A	–	–
Inositol	–	–	–	–
Inulin	–	–	–	–
Maltose	A	A	–	–
Mannitol	A	A	–	–
Mannose	A	A	–	–
Lactose	–	–	–	–
Rhamnose	–	–	–	–
Salicin	–	–	–	–
Sorbose	–	–	–	–
Sucrose	A	A	–	–
Sorbitol	–	–	–	–
Trehalose	A	A	–	–
Xylose	–	–	–	–
Sensitivity to 0/129	–	–	–	+
Novobiocin	–	–	+	–
Hugh-Leifson Test:				
Open	+	+	–	–
Sealed	+	+	–	–
Nitrate reduction	+	+	+	+
KCN broth	+	+	–	–
Ammonia	+	+	+	+

K = Alkaline
A = Acid
sl+ = slightly positive
nt = not tested

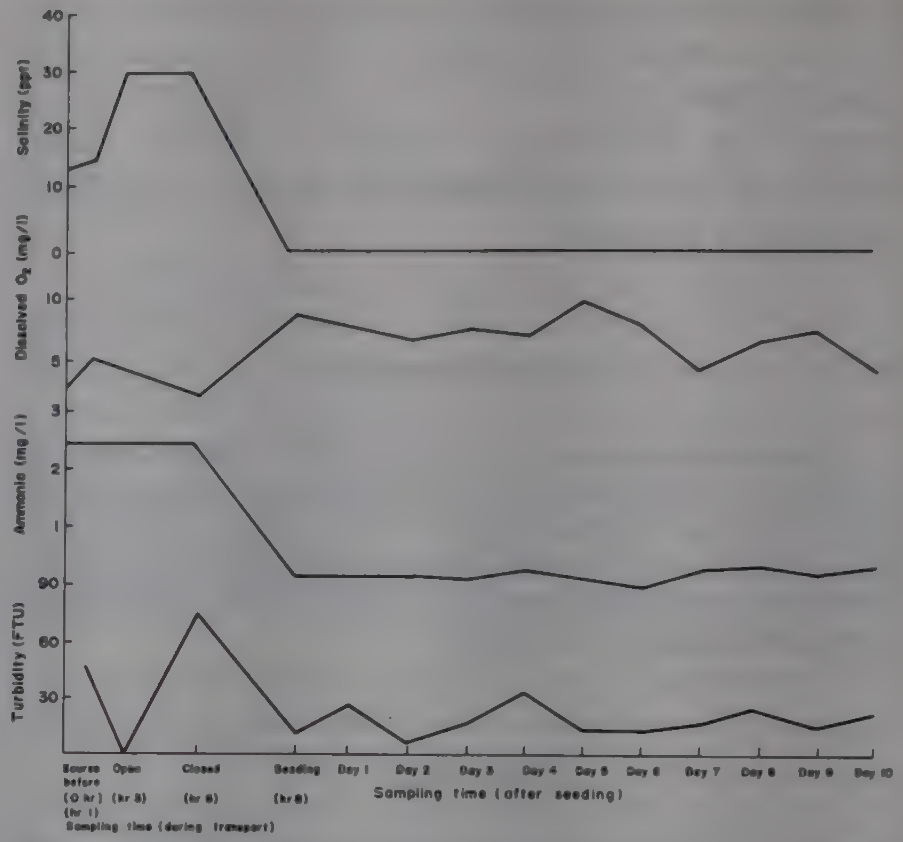

Fig. 1. Physicochemical parameters of water medium of milkfish fingerlings before and during transport by boat and after stocking in Laguna Lake pens.

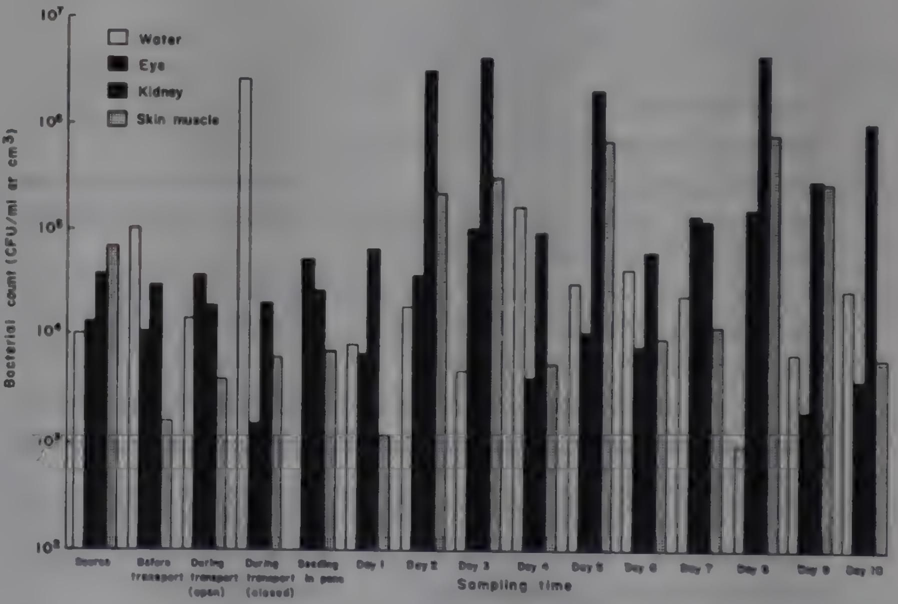

Fig. 2. Mean bacterial counts (CFU/ml or cm^3) of fish organs and water medium of milkfish fingerlings transported by boat and stocked in Laguna Lake pens.

Aeromonas hydrophila Infections in Thailand

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Abstract

Aeromonas hydrophila, synonyms *A. punctata* and *A. liquefaciens*, is a free-living bacterium which causes disease in fishes. The organism is considered an opportunistic pathogen for man. This paper reviews *A. hydrophila* infections, with special reference to current knowledge and work in Thailand in terms of bacteriology, immunology, clinical features, therapeutics and epizootiology of the diseases in both man and animals. Apparently, the first reported occurrences of *A. hydrophila* infections in animals and man in Thailand were in 1976 and 1979, respectively. The main features of disease in fishes were erosion of scales, fin rot, gill rot and ulcerations. Mortality was 0-20%. In human infection, diarrhea is most prominent. Most of the septicemia patients suffer from underlying diseases, such as leukemia, aplastic anemia and cirrhosis. For treatment of infections, sulfas and antibiotics are typically recommended. The characteristics of *A. hydrophila* were quite typical. The organism produced various toxins, e.g., enterotoxin, dermonecrotic toxin, cytotoxin and hemolysin. The bacterin, under laboratory trials, could protect fish against the infection. However, the organism, according to the somatic antigen, could not be classified. Drug susceptibility tests indicated that the strains from fishes were more resistant to antibiotics than were the human isolates.

Distribution

It is well known that *Aeromonas hydrophila* is ubiquitous in natural waters in all parts of the world. Under certain conditions, the organism can cause diseases in aquatic animals, as well as in humans. In Thailand, undiagnosed mortalities of clariid catfish *(Clarias batrachus)* showing square hemorrhagic ulcerations occurred frequently during the early days of clariid catfish culture. Saitanu et al. (1976) demonstrated that *A. hydrophila* was the causative pathogen. Three years later, human infection was reported (Lelaratsmi et al. 1979).

Knowledge of the ecology of this pathogen in Thailand was very poor in the past. It was not until 1983 that the organism was isolated from water in which fish suffered from the disease, as well as in areas where disease did not occur. The number of *A. hydrophila* in the infected areas was usually as high as 10^3-10^5 cells/ml (Saitanu and Poonsuk 1983, 1984). It was indicated that high numbers of this pathogen were related to the outbreak of the disease (Saitanu 1985a). Nevertheless, the organism could be isolated from healthy people (Pitarangsi et al. 1982; Kositanont and Phan-Urai 1983; Sukroongreung et al. 1983). It is worth noting that during the fish epizootic of 1983, 16% of 25 asymptomatic people carried the organism in their stools (Reinprayoon et al. 1984).

Characteristics

Seventy biochemical, physical and morphological properties were studied in 92 strains of *A. hydrophila* and two strains of *A. salmonicida* (Saitanu and Poonsuk 1980a). The organisms showed typical characteristics of the reference strains. *Aeromonas hydrophila* tolerated 4% NaCl, grew at various pHs (4.7-11.0) and temperatures (10-42°C) (Sihanonth et al. 1983a; Tanasupawat and Saitanu 1984). It produced several extracellular enzymes that hydrolyze starch, casein, DNA, gelatin, red blood cell, serum and tween-80 (Tanasupawat and Saitanu 1985). Using the IMVC tests, Sukroongreung et al. (1983) could divide the organisms isolated from different sources into 11 biogroups.

A. hydrophila isolated from fish produced heat labile toxin and killed mice (Tesprateep et al. 1983). Most strains from fish produced hemolysin (Laohaviranit 1983; Chaiprasittigul and Kusamran 1984, Laohaviranit and Vongthongsri 1984), cytotoxin (Laohaviranit 1983; Laohaviranit and Vongthongsri 1983; Supawat et al. 1983) dermonecrotic factor and enterotoxin (Luangtongkum et al. 1983, 1985). The toxic activity was decreased by acid, high pH and heat. Proteolytic, hemolytic and cytotoxic activities were completely destroyed after heating to 100°C for 10 min. (Laohaviranit 1983). Wanishwattana and Kusamran (1984) found that the hemolysin-producing strains of *A. hydrophila* contained plasmids.

Diseases

A. hydrophila was first isolated from the stool of a dead white-handed gibbon and the brain of swine. The primary clinical sign in the gibbon was diarrhea while incoordination and circular walking was observed in pig (Saitanu et al. 1976). Table 1 shows the affected aquatic animals in which *A. hydrophila* was the primary cause. Mortality in infected clariid catfish was 0-20%. The typical lesions were square hemorrhagic ulcerations in catfish, gill rot and scale erosion in sand goby and snakehead fish, ascitis and hemorrhage in carp and gold fish, deep ulcers and abdominal hemorrhage in swamp eel. During the epizootics of 1982-1983 and the following years. *A. hydrophila* was found to cause secondary infections in most species of scaled-freshwater fishes (Boonyaratpalin et al. 1983; Poonsuk et al. 1983; Booranasawettathrum et al. 1984; Menasveta 1985; Tonguthai 1985).

The first infection in man in Thailand was reported in 1979 (Lelaratsmi et al. 1979). The patients had underlying diseases, mostly cirrhosis and malignancies. Since that time, several additional reports of such secondary infection have appeared (Lelaratsmi and Aswapokee 1979; Thamlikitkul and Danchaivijitr 1981; Aswapokee et al. 1983a, 198b; Limsuwan 1984). The association of the organism with diarrhea was in doubt (Pitarangsi et al. 1982). However, there were several reports of the isolation of *A. hydrophila* from diarrheal patients (Vibulbandhitkij et al. 1982; Kalnauwakul and Rutanarugsa 1983; Nilakul et al. 1983; Sutra et al. 1984). Chumkasien (1983) found that during the fish epizootic of 1982-1983, cases of diarrhetic patients caused by *A. hydrophila* were markedly increased. Likewise, Reinprayoon et al. (1985) reported that diarrheal patients suffered from consuming contaminated water and fish.

Saitanu et al. (1976) showed that a dose of 8×10^6 cells of *A. hydrophila* could produce a typical square hemorrhagic lesion in clariid catfish 24 hours after intramuscular injection. It was demonstrated that trash fish, casamino acid and a low temperature, 22^oC, elevated the virulence of the organism (Mahamontri et al. 1983; Sihanonth et al. 1983b).

Chemotherapeutics and Chemoprophylaxis

Antibotics and sulfas have been used for therapeutics and prophylaxis against *A. hydrophila* in cultured fish in Thailand for more than 15 years. Generally the combination of drugs for treatment was more effective than a single chemical (Saitanu and Chalarak 1983; Saitanu and Poonsuk 1984). However, sensitive drugs were found to be equally effective against either experimental or natural infection (Tangtrongpiroj Wongsatayanon 1985; Vorapongsittikul et al. 1985). Mektrairat et al. (1985) showed that tetracycline, 5 mg/kg, I/P, could protect fish against infection for four days. Long-term application of antichemotherapeutic agents created drug-resistant *A. hydrophila* which could be isolated from fish. Poonsuk and Saitanu (1980) found that the drug-resistance pattern was different from place to place, depending on the history of drug application. The organism was more resistant to sulfas than to antibiotics (Navarat et al. 1979; Saitanu et al. 1979; Saitanu and Poonsuk 1980c; Saitanu and Wongsawang 1982; Reungprach and Kesornchandra 1983). The study of minimal inhibitory concentrations of various drugs to *A. hydrophila* was undertaken by Poonsuk et al. (1984, 1985a, 1985b).

During the fish epizootics of 1982-1983, some disinfectants for the eradication of *A. hydrophila* in fishponds were recommended. To shed more light on this field, the effect of various kinds of disinfectants (e.g., potassium permanganate, formalin, benzalkonium chloride, iodine) was also studied (Tachushong and Saitanu 1983a, 1983b; Saitanu 1985b; Saitanu and Yingyong 1985; Saitanu et al. 1985). The bactericidal activity of the disinfectants deteriorated when exposed to organic matter. As to drug susceptibility, most strains of *A. hydrophila* from humans were highly sensitive to antibiotics and sulfas (Nilakul et al. 1984; Reinprayoon et al. 1984).

Vaccine Development

Some viral and bacterial diseases in fish can be prevented by vaccination. A vaccine for preventing *A. hydrophila* infection is under laboratory trials. Clariid catfish produced an agglutinating antibody after being vaccinated with bacterin prepared from a strain of *A. hydrophila* (Saitanu and Poonsuk 1979; Saitanu et al. 1982b; Kesornchandra and Boonyaratpalin 1985). Likewise, snakehead fish responded to vaccination (Obsuwan et al. 1982). The vaccinated clariid catfish, under laboratory trials, were protected from the homologous strain of *A. hydrophila* (Saitanu and Poonsuk 1980b). Because the organism is very heterogeneous according to the o-antigen serogroup study (Saitanu et al. 1982c) to prevent the disease, multivalent vaccine must be prepared.

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Table 1. *Aeromonas hydrophila* infection as a primary cause in aquatic animals.

Affected species	Scientific name	References
Clariid catfish	*Clarias batrachus*	Saitanu et al. 1976
Striped catfish	*Pangasius sutchi*	Tanasomvang and Saitanu 1979
Sand goby	*Oxyeleotus marmoratus*	Saitanu et al. 1979
Swamp eel	*Fluta alba*	Navarat et al. 1979
Snakehead fish	*Channa striata*	Saitanu and Poonsuk 1980c
Frog	*Rana tigrina*	Wongsawang et al. 1982
Carp	*Cyprinus carpio*	Saitanu and Wongsawang 1982
Gold fish	*Carassius auratus*	Saitanu et al. 1982a
Giant goramy	*Osphronemus goramy*	Saitanu et al. 1982a
Pompadour fish	*Symphysodon aequifasciapa*	Saitanu et al. 1982a
Softshell turtle	*Trionyx cartilagineus*	Saitanu 1985a

Bacteriological Examination of Fingerlings of Bighead Carp *(Aristichthys nobilis)* and Grass Carp *(Ctenopharyngodon idella)* Imported into Malaysia

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Abstract

Six genera of bacteria potentially pathogenic to fish were isolated from the organs of *Aristichthys nobilis* and *Ctenopharyngodon idella* fingerlings obtained from two fish importers of Malaysia during the five-month study. The organs examined were the kidney, liver, intestine and gills. The methods and media used enabled the isolation of aerobic, heterotropic mesophiles in the organs of the fingerlings.

A large number of the isolates were gram negative rods, of the genera *Aeromonas, Proteus, Citrobacter, Pseudomonas, Flavobacterium* and *Chromobacterium*, found by other workers to be potentially pathogenic to fish. Several isolates of gram positive cocci of the genera *Micrococcus* and *Staphylococcus* were also identified and could also be pathogenic to fish. The genus *Bacillus*, described as nonpathogenic to fish, was also isolated.

In a reinfection trial, *Aeromonas, Citrobacter* and *Pseudomonas* were found to be pathogenic when injected intraperitoneally into healthy fingerlings of the grass carp. *Aeromonas, Pseudomonas, Enterobacter* and *Flavobacterium* were also isolated from the kidney and liver of locally-bred fingerlings of bighead and grass carp.

Introduction

Bacteria are common infectious agents of fish disease and can cause heavy mortalities of both cultured and wild fish (Shotts and Bullock 1975; Hambal 1985; Menasvata 1985). Bacteria are either obligate or facultative pathogens. Facultative pathogens can be a potential threat when fish are under environmental and physiological stress (Wedemeyer 1970).

In Malaysia, very little is known about the bacteriology of fish. The only available data on *Vibrio* sp. were from marine fish during occurrences of disease and severe mortalities (Ong and Wong 1985) and on *Pseudomonas* sp. isolated by Shariff and Law (1980) from diseased freshwater fish in Johore, Malaysia.

Aquaculture is an expanding industry in Malaysia and this increase could give rise to deterioration of optimal aquatic environments for fish culture. Intensive aquaculture as seen in Thailand and Indonesia has resulted in epizootics due to the facultative pathogen *Aeromonas hydrophila*.

This bacteriological study of imported bighead *(Aristichthys nobilis)* and grass carp *(Ctenopharyngodon idella)* was carried out as an initial step to establish basic information on the bacterial flora of fish in Malaysia. The bacterial flora of imported fry was studied because more than 50% of cultured fish in Malaysia are grown from fry imported from other parts of Asia. Information on the various potential pathogenic bacteria found in cultured fish can provide information to develop preventive, control, and treatment methods against future bacterial epizootics.

Materials and Methods

Fingerlings of *A. nobilis* and *C. idella* imported from Taiwan were purchased from two main Malaysian importers, The South East Asian Farm, Enggor, Perak and Ban Lee and Company, Salak South, Selangor. One hundred and five *A. nobilis* and 105 *C. idella* over 5 months old, average length 7 cm and 9 g weight were sampled for bacteria. The importing agencies maintained the fingerlings in earth ponds or concrete tanks before selling them.

Thirty-five locally-bred fingerlings, 16 *A. nobilis* and 19 *C. idella*, were also sampled for bacteria. These fingerlings were obtained from the Freshwater Fishery Research Station, Batu Berendam, Melaka.

The fish were transported to the laboratory at Universiti Pertanian Malaysia in oxygenated plastic bags and bacteriological sampling was carried out upon arrival.

The fish skin was washed with 70% ethanol to reduce the number of incidental organisms. An incision into the ventral abdomen was made through the body wall with a sterile scalpel at the mid-ventral line. The liver was sampled by first placing a hot scalpel blade on the surface and then inserting a flamed straight wire into the organ. The kidney was sampled similarly by inserting a flamed straight wire, but the surface was not seared with a hot blade as the kidneys were too small. The materials obtained from these organs were streaked directly onto plates of trypticase soy agar (TSA, Difco).

Samples of gills and intestines were taken by excising a small portion of the organ, dipping it in 70% ethanol and transferring it into phosphate buffered saline (PBS) at pH 7.4. Pools of gills and intestines were homogenized separately and surface-plated onto TSA.

All inoculated plates were incubated aerobically at 22°C. After 48 hours, representative numbers of the most numerous colony types from each plate were picked and subcultured repeatedly until pure cultures were obtained. The colonies were maintained on TSA slant at 4°C.

All purified bacterial isolates were characterized to genus and species level by examination of the colony morphology and biochemical characteristics using API 20E Enterobacteriaceae identification kit. Methods and media as described in Cowan and Steel (1974) were used as supplementary tests to reconfirm the identity.

Preliminary identification tests were conducted to study the colony morphology and pigmentation, cell shape and arrangement, gram stain and motility of the cells, as well as the ability of the isolate to produce oxidase and catalase enzymes. The oxidative or fermentative nature of the isolates was determined with glucose (1% w/v) in Bacto OF basal medium and the test for acid and gas production from glucose (1% w/v) was conducted in the Andrade Peptone Water medium (Oxoid).

Supplementary tests were employed to reconfirm the identity of the isolates as described in Cowan and Steel (1974), which included indole production, citrate utilization, growth in triple sugar iron (TSI) agar, MR-VP, urease and gelatinase activity.

The identification of gram positive bacteria was based only on gram reaction, colony morphology, cell shape and arrangement, production of catalase and oxidase and motility.

The virulence of 10 isolates representing five genera was tested. Fingerlings of *C. idella* were used and maintained in aquaria filtered with undergravel filters at water temperatures of 27-29°C.

A pure culture of each isolate was grown overnight in trypticase soy broth at 22°C with aeration. The culture was then centrifuged at 4,000 rpm for 20 min. and the cells resuspended in PBS solution. *C. idella* fingerlings, 9 g average weight were injected intraperitoneally with 0.05, 0.1 and 0.2 ml of the corresponding suspension of each bacterial isolate. Mortalities were recorded daily during a 7-day period. Death due to the injected bacterial strains was confirmed when pure culture of the injected bacterial strains were reisolated.

Results

Tables 1, 2, 3 and 4 show the bacterial isolates identified from imported *A. nobilis* and *C. idella* fingerlings. A total of 470 bacterial isolates were associated with the organs of both fish species and these isolates comprised 12 different gram negative genera. Four of these bacterial types, *Aeromonas hydrophila, Citrobacter freundii, Enterobacter* sp. and *Pseudomonas* sp. were isolated in both *A. nobilis* and *C. idella* obtained from the two importing agencies. Three other bacterial groups, identified as *Proteus* sp., *Acinetobacter* and *Plesiomonas shigelloides,* were present in three of the four groups of imported fish, i.e., *C. idella* and *A. nobilis* from the two importing agencies. Other bacterial groups, *Escherichia coli, Erwinia* sp. and *Klebsiella* sp. occurred at a lower prevalence than the seven bacterial groups mentioned earlier. *Flavobacterium* sp. and *Chromobacterium* sp. were isolated only in fingerlings of grass carp purchased from Salak South.

Gram positive groups belonging to the genera *Bacillus, Staphylococcus* and *Micrococcus* were also isolated. Specific determination was not done on these groups. The identities of these isolates were presumed on the basis of gram reaction, cell shape and arrangement, colony morphology and pigmentation, motility, catalase and oxidase test.

Among the gram negative group, *A. hydrophila,* followed by *C. freundii,* were the most frequent isolates and were detected on both host species from both importing agencies. A consistently large number of *A. hydrophila* isolates were found in each of the organs in all instances. In addition, gram positive groups were also frequently isolated in all organs of both host species from Salak South and Enggor. Ten other generic groups were not consistently distributed in all organs and their prevalence were lower than *A. hydrophila,* gram positive genera and *C. freundii.*

Bacteria of four different groups were isolated from locally-bred fingerlings (Table 5). Three of these four groups, *A. hydrophila, Enterobacter* sp. and *Pseudomonas* sp., were common in both local and imported fingerlings of *A. nobilis* and *C. idella.*

Test on the pathogenicity of the bacteria *A. hydrophila, P. aeuriginosa, Proteus vulgaris, C. freundii* and *Enterobacteriaceae* showed that all isolates caused death of fingerlings at various times after experimental infection.

At 0.05 ml inoculum, strains of *P. aeuriginosa* and *A. hydrophila* killed all 10 fingerlings within one and two days, respectively. A mortality of more than 50% occurred after 2-3 days among fingerlings injected with 0.1 ml isolates of *C. freundii, P. vulgaris* and *Enterobacteriaceae.* The control group injected with PBS showed 100% survival. One isolate, *P. mirabilis,* was not pathogenic to *C. idella* fingerlings.

Discussion

As this was a preliminary study of bacterial flora in imported and locally-bred fingerlings, a non-selective medium (TSA) was used which allowed only for the recovery of a limited number of bacterial groups. The bacteria types isolated were all aerobic, heterotrophic and mesophilic. Diverse genera of gram negative bacteria were detected in the internal organs of the imported bighead and grass carp. Shotts et al. (1976) described similar bacterial flora from aquarium fishes imported from Southeast Asia into Canada. Three of the genera: *Aeromonas*, *Pseudomonas* and *Flavobacterium*, but of different species, were also isolated from healthy and diseased grass carp reared in freshwater ponds in Taiwan (Wu 1971). The species *A. punctata*, *A. sobria* and *P. fluorescens* and other organisms were isolated from diseased grass carp in China (Nei and Pan 1985).

Clusters of gram positive cocci were isolated and their identity presumed to be *Micrococcus*. This genus was also detected in healthy and diseased grass carp in Taiwan (Wu 1971). *Staphylococcus* sp. was also recovered occasionally in organs of the imported fingerlings. This genus is known to have caused epizootics in some fish species (Kusuda and Sugiyama 1981).

The detection of the gram negative isolates *A. hydrophila*, *Pseudomonas* sp., *Proteus* sp. and *Flavobacterium* is significant because these are known to be potential fish pathogens (Bullock 1971; Frerichs 1984). *Aeromonas hydrophila* was the most dominant isolate in all organs of both bighead and grass carp and was detected in all samples over the five-month period. This organism is one of the main opportunistic pathogens for both freshwater and estuarine fishes (De Figueiredo and Plumb 1977; Nieto et al. 1984). Aeromonads were the main etiological agent in a disease outbreak in Indonesia where severe mortality of both cultured and wild fish resulted (Hambali 1985). Several workers in Thailand (see Menasveta 1985) confirmed that *A. hydrophila* was a major bacterium isolated from the ulcerative lesions of fish from ponds and the wild during this disease epizootic. The occurrence of serious disease due to *A. hydrophila* in countries so near Malaysia indicates the need to monitor its distribution and occurrence in Malaysian fish. The other gram negative organism *(Proteus vulgaris*, *Pseudomonas putida*, *Citrobacter freundii* and *Chromobacterium* sp.) which were occasionally isolated from organs of these imported fingerlings should be a cause of concern as well. *Proteus vulgaris* has been known to cause blotch disease in some species of freshwater fishes (Amlacher 1961) and *Pseudomonas putida* is one of the etiological agents of hemorrhagic septicemia in fish (Bullock 1971). In addition, *Citrobacter* sp. and *Chromobacterium* sp. have been isolated from ulcerated lesions of fish in Papua New Guinea (Coates et al. 1984).

The potential pathogenic nature of five of the gram negative isolates was seen in the pathogenicity study. Thus, the strains of bacteria isolated from bighead and grass carp fry imported into Malaysia could presumably become pathogenic under stressful situations.

The bacteriological examination of locally-bred fingerlings showed the presence of four bacterial types similar to the ones isolated from imported fry, namely, *A. hydrophila*, *Enterobacter* sp., *P. aeuriginosa* and *Flavobacterium*. Further studies would be required to identify the bacterial flora present on the local freshwater fish before it could be concluded that potential disease-causing bacteria are being introduced via imported fish.

The number of bacterial types isolated in this study do not represent all the organisms found in the organs of imported fingerlings because sampling was carried out for only five months. Two potentially pathogenic genera, *Flavobacterium* sp. and *Chromobacterium* sp., isolated only in grass carp fingerlings purchased from Salak South, would probably be isolated in other samples if an additional seven-month sampling is carried out. Furthermore, 12-month sampling would allow the recovery of bacterial types predominant in different seasons of the year.

In conclusion, this preliminary study shows that there are potential pathogens present as part of the normal bacterial flora of both imported and locally-bred fingerlings. However, the strain types of these pathogens still have to be determined to see if the strains found here are similar to those known to cause severe mortality of fish in countries such as Thailand and Indonesia. Monitoring the entry of these pathogenic strains through imported fingerlings would help prevent bacterial epizootics of fish in Malaysia. Furthermore, if strain types in local fingerlings are known, the entry of new strains with imported fingerlings could be restricted, preventing possible epizootics due to local fingerlings not being resistant to new strains.

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Table 1. Prevalence and distribution of bacterial groups in organs of *A. nobilis* from Salak South, Selangor (n = 105).

Bacteria	No.	Distribution of isolates in various organs Kidney	Liver	Gill	Intestine
Aeromonas hydrophila	53	23	17	7	6
Citrobacter freundii	26	5	5	8	8
Enterobacter cloacae	2	2	—	—	—
Pseudomonas mallei	1	—	—	1	—
Proteus vulgaris	7	6	1	—	—
Escherichia coli	1	—	—	—	1
Erwinia sp.	1	—	—	—	1
Acinetobacter sp.	1	—	—	—	1
Klebsiella sp.	1	—	—	—	1
Gram positive	30	2	2	18	8
Unidentified gram Negative	8	—	4	1	3
Total isolates	131	38	29	35	29

Table 2. Prevalence and distribution of bacterial groups in organs of *A. nobilis* from Enggor, Perak (n = 105).

Bacteria	No.	Distribution of isolates in various organs			
		Kidney	Liver	Gill	Intestine
Aeromonas hydrophila	57	15	20	6	16
Citrobacter freundii	13	4	3	2	4
Enterobacter cloacae	2	—	—	—	2
Pseudomonas aeuriginosa	3	—	—	—	3
Proteus vulgaris	3	—	—	—	3
Proteus mirabilis	2	1	1	—	—
Plesiomonas shigelloides	1	—	1	—	—
Gram positive	26	3	4	14	5
Unidentified gram Negative	6	2	2	1	1
Total isolates	113	25	31	23	34

Table 3. Prevalence and distribution of bacterial groups in organs of *C. idella* from Salak South, Selangor (n = 105).

Bacteria	No.	Distribution of isolates in various organs			
		Kidney	Liver	Gill	Intestine
Aeromonas hydrophila	43	12	16	5	10
Citrobacter freundii	12	2	4	1	5
Enterobacter cloacae	9	1	4	1	3
Pseudomonas aeuriginosa	9	1	1	3	4
Proteus vulgaris	5	5	—	—	—
Plesiomonas shigelloides	2	—	1	—	1
Flavobacterium meningosepticum	2	1	—	1	—
Acinetobacter sp.	1	—	—	—	1
Chromobacterium sp.	6	3	—	2	1
Gram positive	38	7	6	18	7
Unidentified gram Negative	6	1	1	1	3
Total isolates	133	33	33	32	35

Table 4. Prevalence and distribution of bacterial groups in organs of *C. idella* from Enggor, Perak (n = 105).

Bacteria	No.	Distribution of isolates in various organs			
		Kidney	Liver	Gill	Intestine
Aeromonas hydrophila	30	8	11	7	4
Citrobacter freundii	14	3	4	3	4
Enterobacter cloacae	8	—	1	—	7
Pseudomonas aeuriginosa	3	1	—	1	1
Pseudomonas putida	3	—	—	2	1
Proteus sp.	0	—	—	—	—
Acinetobacter sp.	1	1	—	—	—
Plesiomonas shigelloides	1	1	—	—	—
Gram positive	30	3	9	14	4
Unidentified gram Negative	4	2	—	—	2
Total isolates	94	19	25	27	23

Table 5. Genera of bacteria from organs of locally-bred fingerlings of *A. nobilis* (n = 16) and *C. idella.*

	No. of isolates	Distribution in organs	
		Kidney	Liver
Aeromonas hydrophila	8	4	4
Enterobacter sp.	4	1	3
Pseudomonas aeuriginosa	4	1	3
Flavobacterium sp.	1	—	1
Unidentified gram negative	4	1	3
Total isolates	21	7	14

Table 6. Pathogenicity of selected bacterial strains isolated from imported *C. idella* and *A. nobilis.*

Strains	Dose of bacteria injected			Time from infection to death (days) with		
	0.05	0.1	0.2 ml			
	Dead fish (n = 10)			0.05	0.1	0.2 ml dose
Aeromonas hydrophila						
SBK8-9	2	6	NT	1	1	
SBK9-12	10	9	NT		2	2
SGL8-16	NT	10	10		1	1
SBL9-10	NT	NT	9			
Pseudomonas aeuriginosa						
SGI8-3	10	10	NT	1	1	
SGI10-12	NT	4	6		3	1
Enterobacteriaceae	NT	9	9		3	4
Proteus mirabilis	0	0	NT	No deaths		
Proteus vulgaris	NT	5	10		3	1
Citrobacter freundii	8	10	NT	2	2	
Control (PBS)	0	0	0	No deaths		

NT = not tested.

The Susceptibility of Various Fish Species to Infection by the Bacterium *Aeromonas hydrophila*

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SUPRIYADI, H. 1986. The susceptibility of various fish species to infection by the bacterium *Aeromonas hydrophila*, p. 241-242. *In* J.L. Maclean, L.B. Dizon and L.V. Hosillos (eds.) The First Asian Fisheries Forum. Asian Fisheries Society, Manila, Philippines.

Abstract

Testing of fish species for resistance against *Aeromonas hydrophila* was conducted. Two strains of common carp *(Cyprinus carpio)*, Taiwan and Sinyonya, and two other species of cultured fish, walking catfish *(Clarias batrachus)* and giant goramy *(Osphronemus goramy)*, were challenged by intraperitoneal injection with three levels of viable pathogen: 10^3, 10^5 and 10^7 bacterial cells per fish. The results indicated that walking catfish was most susceptible to infection by *Aeromonas hydrophila*. Giant goramy was more resistant than walking catfish but less resistant than both the Sinyonya and Taiwan strains of common carp which did not differ in susceptibility. Comparison of mortality rates for the different levels of pathogen used to challenge fish showed that mortality was highest at 10^5 bacteria per fish. However, fish injected with 10^3 bacterial cells were less severely infected than those challenged with 10^7 bacterial cells.

Introduction

Fish culture in Indonesia, such as through the use of running water systems, has intensified since 1971. Type of culture system, nutrition, broodstock quality, water quality and disease are factors to be considered in establishing fish culture. Disease is one factor which reduces fish production. Outbreaks of fish caused great damage to carp production in late 1980 in West Java, where a total of 125 t of carp were lost (Djajadiredja et al. 1983).

Some antibiotics and chemicals have been successfully used to treat fish disease. The addition of antibiotics to fish feed is one of the most useful methods of treating fish against *Aeromonas* sp. or *Flexibacter columnaris* infections (Meyer 1964; Curran and Herman 1969). However, these methods of treatment are limited to fish accustomed to artificial diet. Each antibiotic has advantages, but also disadvantages as some produce drug resistant strains of bacteria when used continuously.

Protection of fish against a number of the more important bacterial diseases is possible by use of vaccines (Horne et al. 1984; Tatner and Horne 1984). The effectiveness of vaccines depends upon their ability to stimulate the immune system of fish so that in the event of natural challenge they are able to mount a quick protective response. Protection resulting from vaccination is usually long lasting; fish require only one or two doses to protect them over a full season of exposure and if vaccines do not cause resistance or residue accumulation which may arise with antibiotics.

Another method of controlling fish disease is by selection of species or strains of cultured fish resistant to disease (Richards and Roberts 1978; Castric and de Kinkelin 1984). Indonesia has over a thousand species and strains of cultured and wild fishes; thus selection of species or strains of fish resistant to disease offers a potentially valuable tool to prevent disease outbreaks.

Results of investigations on the resistance of species and strains of fish to *Aeromonas hydrophila* are presented.

Materials and Methods

Two strains of common carp *(Cyprinus carpio)*, Sinyonya and Taiwan strains, and two other cultured species, giant goramy *(Osphronemus goramy)* and walking catfish *(Clarias batrachus)*, were challenged by intraperitoneal injection with three levels of viable pathogen, 10^3, 10^5 and 10^7 bacterial cells per fish, 10 fish per challenge level. Fish were then fin-clipped to denote the dosage and all fish of the same strain/species held together in the same concrete pond with 10 uninfected fish. There were replications for each fish species or strain. Pond size was 9 x 6 m and 0.8 m deep. Completely randomized design was used in the experiment and the data analyzed with Duncan's Multiple Range Test. Fish were observed for one month and all mortalities recorded.

Bacteria used to test resistance were isolated from infected fish collected from the Bogor area and identified by the methods of Bullock (1971) and Shotts and Bullock (1975).

Results and Discussion

Results indicate that there are differences in susceptibility among the four groups tested (Table 1). Walking catfish was most susceptible to *Aeromonas hydrophila* ($P < 0.01$), giant goramy was more resistant than walking catfish but less resistant than Sinyonya and Taiwan strains of common carp. Susceptibility did not differ significantly between the Sinyonya and Taiwan strains.

The high susceptibility of walking catfish to bacterial disease appeared to be confirmed by several cases of outbreaks in Indonesia. Similar cases among walking catfish have also been reported by Menasveta (1985) and Areerat (1978) in Thailand.

Snakehead *(Ophicephalus striatus)* also seems to be susceptible to *Aeromonas hydrophila*. During outbreaks of bacterial disease in Kalimantan and South Sumatera, this species was seriously infected by this bacterium.

Comparison of results of challenge with different levels of bacteria showed that a dosage of 10^5 bacteria/fish caused higher mortality than 10^7 or 10^3 bacteria/fish. However, a dosage of 10^3 bacteria/fish caused less severe infections than 10^7.

The LD_{50} of *Aeromonas hydrophila* was studied by Supriyadi (1985). The LD_{50} for *A. hydrophila* isolated from different places in Indonesia ranged from 1.5×10^5 to 1.5×10^6. Similarly, this study showed that a challenge of 10^5 bacteria caused high mortality. Snieszko (1974) mentioned that the pathogenicity of some bacteria is influenced by environmental conditions.

Early signs of infection were first observed three days after post-injection. Hemorrhage and necrosis of the skin and occasional ulcerations were observed.

According to a recent study at the Research Institute for Freshwater Fisheries, the crossing of Majalaya and Sinyonya carp strains produces a fast-growing hybrid. The susceptibility of this new hybrid to bacterial disease also needs to be studied in the search for a disease-resistant carp strain.

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Table 1. Mortality of fishes challenged with different levels of *Aeromonas hydrophila.*

Fish species	Levels of viable pathogen (cells/fish)	Trial I	Mortality of fish (%) in replicate Trial II	Trial III
Giant gouramy *(Osphronemus gouramy)*	10^7	18.4	45.0	56.8
	10^5	56.8	50.8	63.4
	10^3	45.0	89.1	26.6
	uninfected	0.9	0.9	0.9
Catfish *(Clarias batrachus)*	10^7	45.0	38.2	63.4
	10^5	71.8	89.1	71.6
	10^3	45.0	63.4	26.6
	uninfected	0.9	0.9	0.9
Common carp Sinyonya strain *(Cyprinus carpio)*	10^7	0.9	0.9	0.9
	10^5	0.9	0.9	0.9
	10^3	0.9	0.9	0.9
	uninfected	0.9	0.9	0.9
Common carp Taiwan strain *(Cyprinus carpio)*	10^7	0.9	0.9	18.4
	10^5	0.0	0.9	0.9
	10^3	0.9	0.9	0.9
	uninfected	0.9	0.9	0.9

Bacterial Flora of Seabass, *Lates calcarifer* Bloch, Imported from Thailand for Cage Culture in Penang, Malaysia

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Abstract

Four groups of seabass, *Lates calcarifer*, imported from Bangkok and Songkla for culture in Penang were examined for the presence of bacteria in the kidney and spleen tissues. Four selective culture media, i.e., TCBS for vibrios, Rimler-Shotts (RS) agar for *Aeromonas*, cetrimide agar for *Pseudomonas*, and cytophaga agar for myxobacteria; and one non-specific culture medium, trypticase soy agar (TSA), were used. Group 1 consisted of healthy fish cultured in Penang for about one month after being imported from Bangkok. The prevalence of bacterial growth on the five media for kidney tissue (spleen tissue) was 11.1% (11.1%) on TSA, TCBS, RS and cytophaga agar and 0% (0%) on cetrimide agar. Group 2 consisted of unhealthy and dying fish imported a day before from Songkla. The prevalence of bacterial growth was 58.5% (65.9%), 24.4% (29.3%), 4.9% (2.4%), 8.1% (8.1%) and 4.9% (0%) on TSA, TCBS, RS, cytophaga and cetrimide agar, respectively. Group 3 comprised diseased fish showing symptoms of tail and fin rot just imported from Bangkok. The bacterial prevalence was 85.7% (92.9%), 50% (50%), 7.1% (7.1%), 7.1% (14.3%) and 0% (0%) on TSA, TCBS, RS, cytophaga and cetrimide agar, respectively. Group 4 was the surviving healthy fish from group 2 examined one month later. The bacterial prevalence was 20% (32%), 12% (18%), 0% (0%), 0% (0%) and 4% (0%) on TSA, TCBS, RS, cytophaga and cetrimide agar, respectively. The unhealthy fish seemed to produce a higher prevalence of bacterial growth on the various media used. The majority of the isolates appeared to be *Vibrio parahaemolyticus* or other vibrios. *Aeromonas*, myxobacteria and *Pseudomonas* were seldom isolated. High prevalence of bacterial growth seemed to be associated with heavy infection by the monogenean *Cycloplectanum epinepheli*.

Introduction

Bacterial diseases are known to cause high mortality of fishes cultured in cages in Malaysia (Chua and Teng 1978; Ong 1984). The annual mortality is known to be between 40 and 50% and the financial loss is considered large because of the high importation cost of fish fry. Many of the seabass fry cultured in the floating cages in Penang are imported by air or by land from Thailand. The fish are normally in poor shape when they arrive, probably due to stress and mishandling during transport. Stress, coupled with lack of a proper quarantine system and effective prophylactic treatment, results in mortality as high as 100% within 1-2 weeks after arrival. Common symptoms associated with this mortality is darkening of the body together with the appearance of a long fecal cast attached to the anus and, occasionally, loss of scales and fin rot.

Diseases caused by bacteria belonging to genus *Vibrio* in marine fish and *Aeromonas* and *Pseudomonas* in freshwater fish have been reported for many food fish cultured in Southeast Asia (Davy and Chouinard 1983; Kabata 1985). There are very few studies of bacterial disease in fish in the tropics. Many studies have shown that bacteria belonging to *Aeromonas*, *Pseudomonas*, *Vibrio*, *Corynebacterium*, *Myxobacterium* and a few others are known to be causative agents of infectious diseases in coldwater fish (Roberts 1978).

In view of the high mortality associated with the importation of fry, this study was initiated and forms part of a larger investigation designed to identify the bacterial pathogens and parasites which may cause death of the imported fish fry.

Materials and Methods

Fish were obtained from the fish farm in Penang in four groups. Group 1 consisted of nine healthy fish cultured in the cage for about a month after importation from Bangkok. Group 2 were 41 unhealthy and dying fish obtained a day after shipment from Songkla. Group 3 were 14 diseased fish showing symptoms of fin and tail rot upon arrival from Bangkok and Group 4 were 25 surviving fish from Group 2 examined one month later.

Live fish were examined within 24 hours for external lesions and abnormalities before they were used for bacteriological examination. The same fish was also used for protozoa and parasite examinations as reported in another paper (Leong and Wong, this vol.). The skin was swabbed with 70% alcohol after the scales had been removed. The abdominal cavity was opened aseptically. Kidney and spleen tissues were removed and inoculated onto five culture media of which thiosulfate citrate bile salt sucrose agar (TCBS) was used to select *Vibrios*, cetrimide (cet) agar for *Pseudomonas*, Rimler-Shotts (RS) agar for *Aeromonas*, cytophaga (cyto) agar for myxobacteria and the nonselective trypticase soy agar (TSA) for all aerobic bacteria, according to the method described by Shotts and Bullock (1975). TCBS was added to the scheme of Shotts and Bullock while 1-3% NaCl was added to the TSA agar for the growth of marine organisms.

All plates were incubated at room temperature (30 2°C) for 24-48 hours and the various colonies selected were transferred to TSA slants for further testing.

The isolates were examined for Gram's reaction, Huge-Leifsons test, oxidase test, catalase test, motility, sensitivity to vibriostatic agent (0/129), methylene blue and novobiocin, MR-VP test, gelatine liquefaction, nitrate reduction, salt tolerance test (0%, 3%, 6%, 8% and 10% NaCl), decarboxylase of amino acids (L-arginine, L-lysine and L-ornithine), growth at 42°C, indole production and luminescence according to the methods described by West and Colwell (1984). Keys for the identification of gram negative aerobic bacteria as described by various authors (Shewan et al. 1966; Gibbs & Skinner 1966; Buchanan and Gibbons 1974) were used and the bacteria identified and grouped under Enterobacteriaceae, *Vibrio*, *Aeromonas*, *Pseudomonas*, *Alcaligenes*, *Flavobacterium*, *Cytophaga/Flexibacter*, *Achromobacter* and other unindentified bacteria. Further identification of species of the Vibrionaceae was done according to the scheme described by West and Colwell (1984).

Results

A total of 89 fish were examined. Fifty-seven (64%) were found to carry bacteria in their kidneys or spleen (Table 1). The occurrence of bacteria depended very much on the health of the fish.

Group 3 fish (diseased) had the highest rate (92.6%) of infection, whereas Group 1 fish (healthy) had the lowest rate (11.1%). Group 4 fish, apparently healthy and from the same batch as Group 2, showed a 44% rate of infection.

The distribution of the bacteria isolated from the four selective media and one general medium is shown in Table 1. Most fish had aerobic bacteria and *Vibrio* in their kidney and/or spleen, as indicated by the high occurrence in the TSA and TCBS media. *Pseudomonas/Aeromonas* and myxobacteria were seldom found.

Sixty-eight isolates from the TSA (Table 2) were found to belong to Family Vibrionaceae and all of them were sensitive to Novobiocin (10μg) and 0/129 at 150μg level. This suggests that all the isolates were vibrios. Eight of the remaining isolates were identified as enterobacteria and nine as other bacteria. The same trend was observed for isolates obtained from TCBS medium. Forty-five out of a total of 54 (83.3%) were identified as vibrios, four (7.4%) as enterobacteria and five (9.3%) as other bacteria. Very few isolates were obtained from the other three selective media and the isolates were mainly enterobacteria (nine), vibrios (two), *Flavobacterium/Cytophaga* group (two), *Alcaligenes/Pseudomonas* group (five) and *Acinetobacter* (one) according to the keys modified from Buchanan and Gibbons (1974), Gibbs and Skinner (1966) and Shewan et al. (1966).

The isolates belonging to Family Vibrionaceae were further identified using the scheme of West and Colwell (1984). Table 3 shows that 38 out of 68 (55.8%) isolates from the TSA medium belonged to Group 1 vibrios that contained *V. parahaemolyticus*, *V. alginolyticus*, *V. harveyi* and *V. vulnificus*. Fifteen isolates (22%) were identified as belonging to Group 2, that contained *V. campbellii*, *V. fischeri*, *V. logei* and *V. marinus*. The remaining 14 isolates (20.8%), as Group 3, were composed of other vibrios needing further identification.

The 45 vibrio isolates obtained from TCBS were further examined with the same scheme of West and Colwell (1984). Twenty-five isolates (55.6%) belonged to Group 1 vibrios; nine (20%) to Group 2 vibrios, and the remaining 11 (24.5%) were other vibrios. Identification of the isolates to the species, however, was difficult due to the many variations among strains of the same species. However, the task of identifying these isolates is still in progress, and may eventually produce a more complete picture of the distribution of vibrios in fish tissues.

Discussion

Results show that TSA with 3% NaCl and TCBS agar are the media most useful and effective in the isolation of bacteria from the kidney and spleen of seabass. The majority of isolates obtained from these two culture media have been identified as vibrios. This agrees with the high number of vibrios in the natural environment where the culture cages are located (Yap 1980; Ong 1984). The occurrence of bacteria in the internal organs of fish has been suggested to be closely associated with the natural flora of the environment (Shewan 1961; Shewan and Hobbs 1967) and was proven by Horsley (1977).

Vibrios are opportunistic organisms present in large numbers in the coastal waters of tropical regions throughout the year. The high prevalence (11-44%) of these organisms in the kidney or spleen of healthy fish supports the view that bacteria of the internal organs reflect the bacterial flora of the environment. Diseased fish harbor an even higher prevalence (78-92%) of vibrios, suggesting that these opportunistic organisms are able to invade and multiply in the tissues of fish under stress and may eventually lead to mortality, as reported by Bullock and Snieszko (1969). The high prevalence of bacteria in the internal organs was reported for many species of fish in the temperate region. Evelyn and McDermott (1961) reported prevalence of 70% in the internal tissues of some 10 species of freshwater fishes. Ojala (1968) isolated bacteria from 49% of healthy fish, whereas 83% of diseased fish harbored bacteria. Bullock and Snieszko (1969) reported 12-28% of fish harbored bacteria and Chung and Kou (1973; 1974) reported 42% of healthy fish and 96.3% of diseased eels were infected. Prevalence in healthy and diseased seabass in this study closely resembles those reported in the literature.

This study reveals that vibrios are the major group of bacteria isolated from fish tissues. This apparently differs greatly from another finding where *Pseudomonas*, *Aeromonas*, *Achromobacter*, *Flavobacterium* and coryneform bacteria predominate (Horsley 1977).

This difference is probably due to different environmental conditions. Most of these studies were done in the cold countries and on fish freshly caught and examined for spoilage bacteria associated with the skin or gills rather than with the internal organs. However, Aiso et al. (1968) reported the isolation of 96.3% vibrios from the intestine of mackerel, *Trachurus japonicus*.

This study shows that vibrios isolated from batches 2 and 3 (healthy and diseased fish) are very heterogenous and contained many species. All these vibrios must have been imported along with the fish. This suggests that disease caused by vibrio infection is probably not the result of a single species but of a combination of two or more. This study indicates that the predominant species of vibrios associated with the kidney or spleen are *V. parahaemolyticus* and *V. alginlyticus*. In Singapore (Chong et al. 1983) and in Malaysia (Ong 1984) vibriosis has been reported to be caused by these species.

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Table 1. Distribution and frequency indices of bacteria isolated from fish on various culture media.

	No. of fish infected/no. examined	Distribution index (% culture)				
		TSA	TCBS	RS	Cyto	Cet
Group 1 (Healthy)	1/9(11.1%)*	11.1(11.1)	11.1(11.1)	11.1(11.1)	11.1(11.1)	0 (0)
Group 2 (Unhealthy, dying)	32/41(78.1%)	58.5(65.9)	24.4(29.3)	4.9(2.4)	8.1(8.1)	4.9(0)
Group 3 (Diseased)	13/14(92.9%)	85.7(92.9)	50.0(50.0)	7.1(7.1)	7.1(14.3)	0 (0)
Group 4 (Healthy)	11/25(44%)	20.0(32.0)	12.0(8.0)	0(0)	0(0)	4.0(0)
Total	57/89(64.0%)					

*Figures without parentheses represent values for kidney, those within parentheses for spleen.

Table 2. Frequency of bacteria isolated on various culture media.

Bacteria	TSA	TCBS	RS	Cyto	Cet
Vibrionaceae	68 (80.0%)	45 (83.3%)	0	0	2
Enterobacteriaceae	8 (9.4%)	4 (7.4%)	2	6	1
Other bacteria	9 (10.6%)	5 (9.3%)	6	2	0
Total	85	54	8	8	3

Table 3. Frequency of vibrio isolates grouped according to West and Colwell (1984).

	Group I	Group II	Group III
TSA	38 (55.9%)	15 (22%)	15 (22%)
TCBS	25 (55.6%)	9 (20%)	11 (24.5%)
Total	63 (56.2%)	24 (21.4%)	25 (22.3%)

Group I Vibrios : *Vibrio parahemolyticus, V. alginolyticus, V. harveyi* and *V. vulificus.*
Group II Vibrios : *Vibrio campbellii, V. fischeri, V. logei* and *V. marinus.*
Group III Vibrios : Unidentified.

Sanguinicola armata Infection in Bighead Carp (*Aristichthys nobilis*) and Grass Carp (*Ctenopharyngodon idella*) Imported in Malaysia

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ANDERSON, I.G. and F. SHAHAROM-HARRISON. 1986. *Sanguinicola armata* infection in bighead carp *(Aristichthys nobilis)* and grass carp *(Ctenopharyngodon idella)* imported in Malaysia, p. 247-250. *In* J.L. Maclean, L.B. Dizon and L.V. Hosillos (eds.) The First Asian Fisheries Forum. Asian Fisheries Society, Manila, Philippines.

Abstract

The first record of the blood fluke *Sanguinicola armata* in *Aristichthys nobilis* and *Ctenopharyngodon idella* in Malaysia is reported. The two species of cryprinids, imported from Taiwan, were examined monthly between February 1984 and February 1985 in Perak and Selangor, Peninsular Malaysia. The average prevalence of *S. armata* adults in all the fish examined was 4.9%. The eggs of *S. armata*, which were readily apparent on examination of gill smears, had an average prevalence of 8.7%. The transparent, lanceolate adult trematodes with spines on their cuticle and their eggs are described. Histopathological examination of a representative sample of *A. nobilis* and *C. idella* revealed the presence of *S. armata* eggs and miracidia in the heart, gills, liver, omentum, kidney, eye, spleen and intestines. The heart and gills were the organs with the highest prevalence at these stages. Adult *S. armata* were seen in the lumen or blood vessels of the heart, liver, kidney, eye and gills. The host response to the various stages of *S. armata* in *A. nobilis* and *C. idella* tissues, which is primarily one of encapsulation at the site of injury, is presented.The distribution of *S. armata* in fish tissues, its potential danger to Malaysian aquaculture and the implications of unrestricted importation of potentially pathogenic parasites are discussed.

Introduction

There is little information available on the parasites of cultured, wild and aquarium fish in Malaysia. Leong (1979) reviewed the then current knowledge on parasites of freshwater fishes in Malaysia listing 24 species, all metazoans. More recently several surveys have added to the knowledge of the known parasites of freshwater fish in Malaysia (Shariff 1980; Shariff and Vijiarungam 1983; Shaharom 1985). Shaharom (1985) reported her findings on imported bighead carp (*Aristichthys nobilis*) and grass carp (*Ctenopharyngodon idella*). These two species comprised 56.64% of total Malaysian freshwater pond aquaculture production in 1983 (Malaysia, Ministry of Agriculture and Fisheries 1984). The use of imported bighead and grass carp fry accounts for all of this significant increase in production.

An examination of imported fishes was initiated to determine primarily the ectoparasites introduced into Malaysia. The survey revealed the presence of triangular-shaped *Sanguinicola* eggs in the gill lamellae of both species examined. This is the first report of the presence of blood flukes in Malaysia. Since there are few detailed histophatological descriptions of the infection, studies were initiated on both the parasite's morphology and its effect on the host.

Materials and Methods

The bighead and grass carp fingerlings used in this study were originally imported from Taiwan as fry. They were purchased monthly from February 1984 to February 1985 from two separate fry importers, one in Salak South, Selangor, and the other in Enggor, Perak. Parasitological examination and sampling for histopathology was carried out within 24 hours of the fishes' arrival at Universiti Pertanian Malaysia.

Fish were killed by transection of the spinal cord. Wet mount smears of the gill lamellae were prepared and examined under the microscope. If the triangular eggs of *Sanguinicola* were detected in the gill lamellae, the heart was then removed from the body cavity, teased open and examined using a dissecting microscope. To relax the trematodes, the adults obtained were held overnight in fish saline at ·5°C. Subsequently they were fixed in 70% ethanol, then stained in paracarmine or a similar stain, destained in acid-alcohol, dehydrated in increasing concentrations of alcohol, cleared in xylene and mounted in Canada balsam for examination with a compound microscope.

Fish were subsampled randomly from several of the consignments for histopathological examination. Fish killed by transection of the spinal cord were then dissected to remove individual organs. Not all organs from every fish were obtained. Fixation was carried out in 10% phosphate buffered formalin and processed using routine methods. Sections cut at 5 to 8um were stained with H + E. Only one section of each organ was examined.

Results

As the heart muscles were teased apart and examined under the microscope, adult parasites wriggled and moved around quickly. Adults were also found in the branchial and caudal blood vessels. *Sanguinicola armata* adults are transparent, lanceolate worms with spines on their cuticular surfaces, averaging 844.65 (370-1,156.20) μm in length and 150.77 (90-232.50) μm in width (n=50). The mouth opens into a long narrow esophagus which ends in a four-lobed intestine. Below the intestine, in the center of the body, arise 10 pairs of testes. On either side of the testes, along the body length are the vitellaria. At the distal end of the testes are a pair of ovaries. Below these are a pair of urinary sacs.

The eggs when newly laid are triangular and measure 44.04 (24.00-52.50) μm, long and 22.00 (16.00-30.00) μm wide. Some of the eggs observed had fully developed oval miracidia with one pair of eyespots and a pair of penetration glands at the anterior end. At the opposite end is a pair of sacs. The miracidium is covered with short cilia.

The percentage prevalence of *S. armata* adults and eggs in all the fish examined over the study period was 3.32 and 6.01, respectively. Table 1 shows the average percentage prevalence in bighead and grass carp purchased from Selangor and Perak, Malaysia.

The egg and miracidial stages of S. *armata* (Fig. 1a) were present in sections of the heart (31/33), gills (33/48), liver (16/45), omentum (12/42), kidney (5/35), eye (2/30), spleen (1/36) and intestine (1/40). None of the 17 brain and 28 skin and skeletal muscle sections contained any stage of the parasite. The percentage prevalence of eggs and miracidia in the organ sections is shown in Fig. 2. The ranking of organs according to percentage prevalence of eggs and miracidia did not differ greatly when bighead carp and grass carp were compared (Fig. 2a), nor when sites of origin in Malaysia (Enggor and Salak South) were compared (Fig. 2b). The number of eggs and miracidia in each organ section examined varied from 111 to 0. The gills and heart had the greatest intensity of infection. Table 2 shows the mean number of eggs and miracidia in relation to organ section.

The adult *S. armata* (Fig. 1b) was less commonly seen in sections. They were detected in the lumen of the bulbus arteriosus (3/33); the posterior vena cava (3/35); the branchial vessels (3/48); the blood vessels within the eye (2/30) and in the hepatic portal vein (1/45).

There was no difference in the response to the presence of the various stages of *S. armata* in bighead and grass carp. The adult parasite was well tolerated and did not evoke any host reaction. The reaction to the egg and miracidial stages was the same and did not vary with the organ. On occasion, there was no host reaction (Fig. 1a). More usually, a reaction in the early stages consisted of encirclement with loosely arranged, irregular, activated fibroblasts (Fig. 1c). This encapsulation appeared to progress to a final stage where there were three to eight concentrically arranged layers of fibrocytes, with collagen fibers occupying the space between the nuclei (Fig. 1d). It was not uncommon to see two or more necrotic eggs encapsulated by the same fibrous connective tissue reaction. A number of stages of destruction of the encysted eggs and miracidia were recognizable in the tissue sections. Unchanged eggs and miracidia were seen occasionally. Most commonly there was a degeneration and necrosis of the eggs and miracidia with macrophages present within the degenerating cell mass (Figs. 1c and 1d). The final stage of destruction was one where the fibrocytic encapsulation surrounded a medulla of faintly eosinophilic, necrotic debris.

Discussion

The parasite *Sanguinicola armata* was identified based on the modified key of Erickson and Wallace (1959) derived from the Macintosh (1934) key. This *Sanguinicola* had a four-lobed intestine and not five as stated by Plehn (1905) for *S. armata*. The rest of the features resemble closely those described for *S. armata*. This is the first reported occurrence of a *Sanguinicola* species in Malaysia and the Southeast Asian region but it is likely that its presence may have been undetected for some time.

The prevalence of adult *S. armata*, as determined by parasitological examination, was higher in grass carp (6.20) than in bighead carp (3.32). Similarly the prevalence of eggs in grass carp (8.06) was higher than in bighead carp (6.01). This is reflected by the higher prevalence of eggs in tissue sections of grass carp as compared to bighead carp (Fig. 2a). The presence of the triangular eggs in gill smears did not necessarily indicate the presence of adults in the bulbus arteriosus. In the sectioned organs and occasionally in parasitological examination, adults appeared to be located in various parts of the circulatory system. Distribution of the adult *S. armata* was comparable to *S. inermis* and *S. klamathesis* in that it was not restricted to the branchial vessels (Bauer et al. 1973; Evans 1974b). Unlike adult *S. davisi* which live only in the gill arch blood vessels (Hoffman 1975). The egg and miracidial organ distribution was wide, with the heart and gills as the most common sites, while the liver, omentum and kidney had lower but notable levels of prevalence. This was more widespread than *S. klamathesis* in cutthroat trout (*Salmo clarki*) where it was reported to be present in the gills, heart and kidneys (Evans 1974b).

These two cyprinids were normal, apparently healthy fish with no signs of disease. The extensive pathology seen in serious disease as described by Bauer et al. (1962, 1973) and Evans (1974b) could not be expected to be seen. In this study the pathology observed was restricted to a host response to the eggs and miracidia. Encapsulation is a typical host response to non-migratory histiozoic helminths (Hoffman 1975). Connective tissue encapsulation of *Sanguinicola* eggs and miracidia has also been described by Bauer et al. (1973), Erickson and Wallace (1959) and Evans (1974b) in a number of different host species. In the *S. armata* infections observed, very large numbers of eggs would need to be present before a severe disease was seen.

Sanguinicola has not been observed in any of the local fish species (Shaharom 1985). The presence of adults and eggs in imported bighead and grass carp fingerlings, without any evidence for the presence of cercarial stages, implies that this parasite is being imported into Malaysia. It is not known if a susceptible snail intermediate host species is present in Malaysia. If it is, then *S. armata* could pose a threat to Malaysian aquaculture as there is no known viable chemotherapeutic agent available to control this parasite and *Sanguinicola* has caused serious mortalities in other parts of the world (Bauer et al. 1962; Evans 1974a). The threat posed by importation of pathogenic parasites to the aquaculture industries of the region via foodfish fry and aquarium fish has been noted before (Shariff 1980; Davy and Chouinard 1983; and Shariff and Vijiarungam 1983). The presence of another exotic parasite in imported cyprinid fry again highlights the need for a quarantine system to prevent the unrestricted distribution of potentially pathogenic parasites, which could become a limiting factor in the development and intensification of aquaculture activities in Malaysia.

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Table 1. Percentage prevalence of *S. armata* adults and eggs from bighead and grass carp purchased from Enggor and Salak South.

	Average % prevalence	Range (%)	Average % prevalence	Range (%)
	Bighead carp n = 240		Grass carp n = 240	
Enggor				
S. armata adults	1.25	0 – 10	6.25	0 – 25
S. armata eggs	6.25	0 – 20	12.50	0 – 45
Salak South				
S. armata adults	5.38	0 – 51	6.15	0 – 20
S. armata eggs	5.77	0 – 30	10.38	0 – 35

Table 2. Mean number of eggs and miracidia in organ sections.

Organ	N	Mean	Range
Gills	48	13.4	0 – 111
Heart	33	10.8	0 – 50
Liver	45	4.6	0 – 52
Eye	30	2.7	0 – 80
Kidney	35	0.7	0 – 13
Omentum	42	0.6	0 – 5
Spleen	36	0.4	0 – 15
Intestine	40	0.025	0 – 1

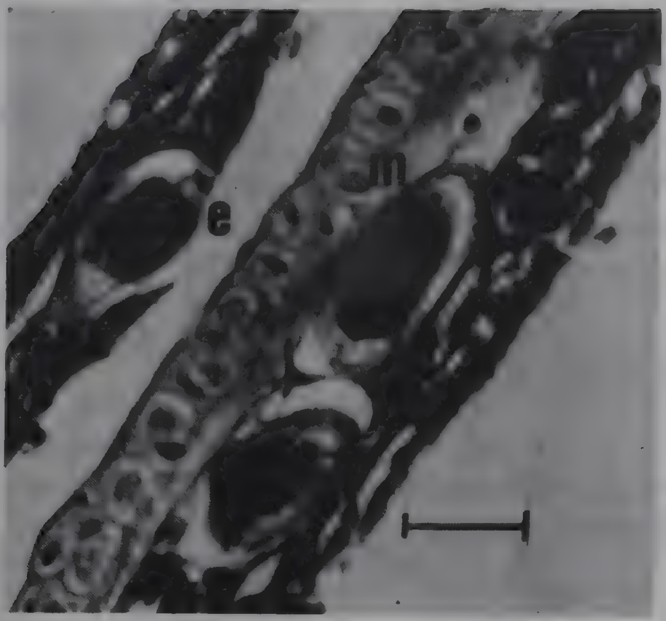

Fig. 1a. Miracidium (m) and egg (e). Gill filament: H+E 400x. Bar = 20 μm.

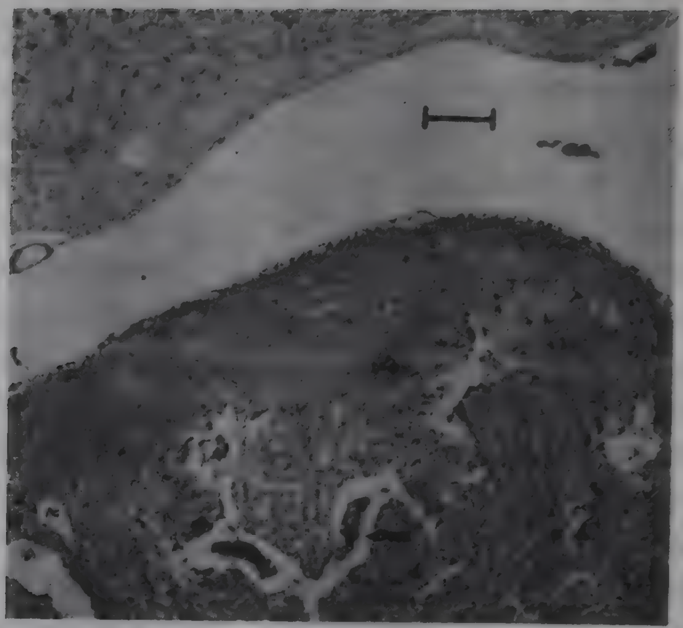

Fig. 1b. Adult *Sanguinicola armata* (arrowed). Bulbus arteriosus: H+E 40x. Bar = 100 μm.

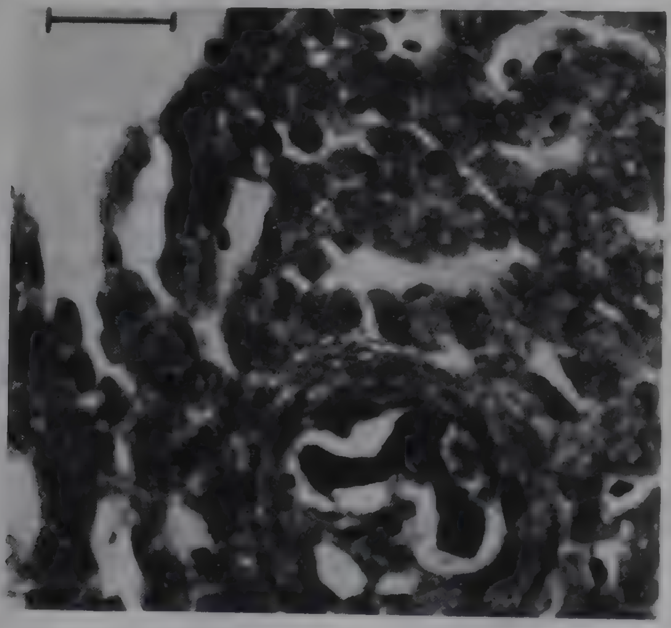

Fig. 1c. Fibroblast encirclement of a degenerating egg. Liver: H+E 400x. Bar = 20 μm.

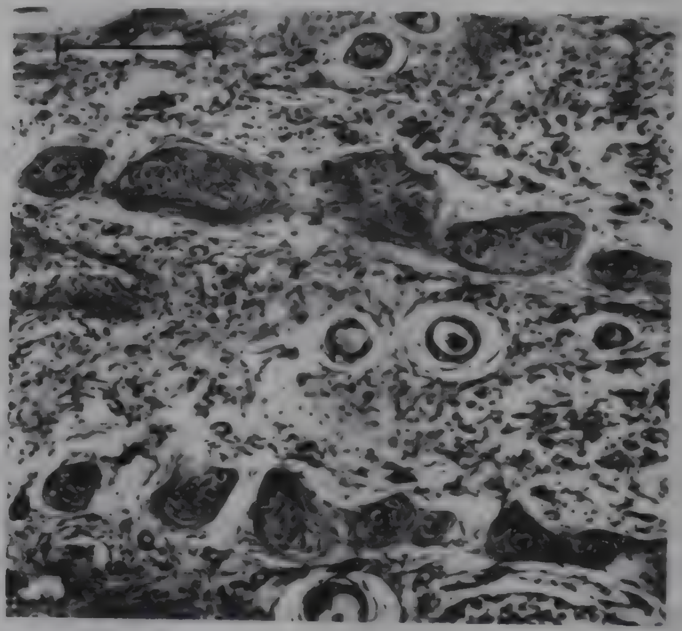

Fig. 1d. Degenerating eggs encapsulated with connective tissue. Gill: H+E 100x. Bar = 100 μm.

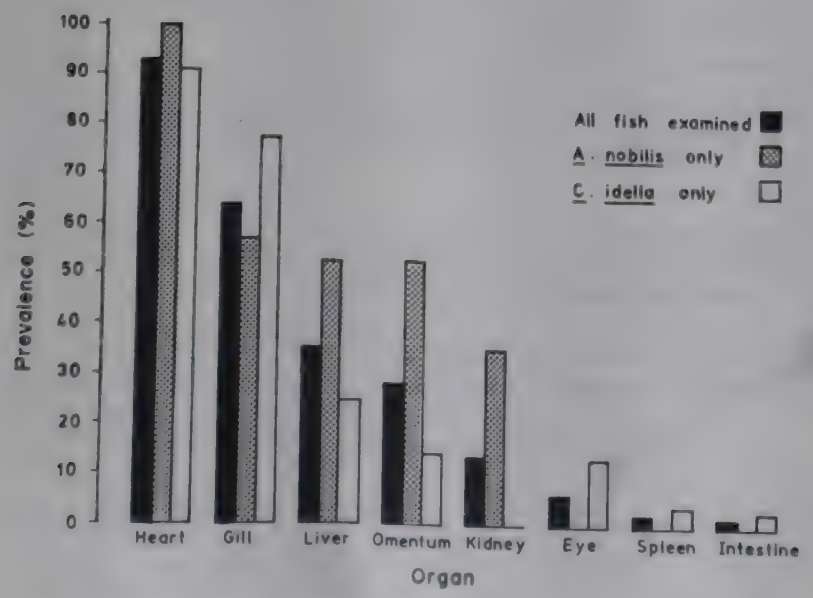

Fig. 2a. Percentage prevalence of *S. armata* eggs and miracidia in relation to organ and species.

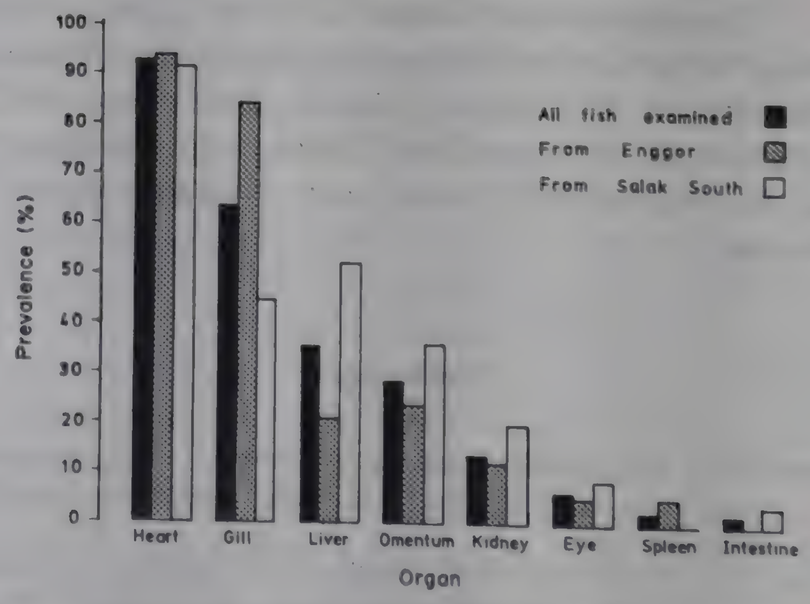

Fig. 2b. Percentage prevalence of *S. armata* eggs and miracidia in relation to organ and Malaysia source.

Parasite Fauna of Seabass, *Lates calcarifer* Bloch, from Thailand and from Floating Cage Culture in Penang, Malaysia

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Abstract

A total of 149 juvenile seabass, *Lates calcarifer* Bloch, 102 imported from Bangkok and 47 from Songkla, Thailand, and 23 market-size fish cultured in Penang, Malaysia were examined for parasites. Seventeen species were identified. These included two Protozoa, two Monogenea, six Digenea, one Cestoda, two Nematoda, two Isopoda, one Copepoda and one Brachiura. Thirteen species of parasites were recovered from fish imported from Bangkok, with an overall prevalence of 90.2%. The monogenean, *Cycloplectanum latesi* Tripathi 1957, was the most common parasite recovered with a prevalence of 78.4% and a mean intensity of infection of 10.8. The nematode *Raphidascaris* sp. also infected a large number of seabass with a prevalence of 40.2% and a mean intensity of infection of 4.9. Five species of parasites were recovered from seabass imported from Songkla, with 95.7% of all fish examined being infected. The monogenean *C. latesi* was the most common parasite encountered, infecting 91.5% of those seabass, with a mean intensity of 14.6. Seabass imported from both Bangkok and Songkla were heavily infected on arrival in Penang and appeared to be weak with darkened body coloration. All parasites found in imported seabass entered Malaysia along with the fish. Twelve species were recovered from market-size seabass collected from Penang. All examined fish were infected with the most frequently recovered parasite, *C. latesi*, with a prevalence of 95.7% and a mean intensity of 83.7.

During pen culture seabass also acquired a large number of parasites, the most common being the digeneans *Pseudometadena celebesensis* (Yamaguti 1952), *Bucephalus* sp., and *Ectenurus* sp. Quarantine procedures are needed to prevent introduction of pathogenic bacteria and parasites into Malaysia.

Introduction

Culture of seabass, *Lates calcarifer* Bloch, in Malaysia depends almost entirely on fry imported from other countries, particularly Thailand. Although Malaysia produces a small number of seabass fry, these are insufficient to meet the needs of the rapidly expanding cage culture industry. This overdependence on imported fry, which are brought into Malaysia without quarantine procedures, can result in the introduction of exotic pathogens. Very often a large proportion of the imported fish have died during the first week or so after release, causing great financial loss to the fish farmer.

Seven species of parasites have been reported from seabass in Thailand: *Cycloplectanum (= Diplectanum) latesi* Tripathi 1957; *Dactylogyrus* sp.; *Lecithochirium* sp.; *Pseudometadena celebesensis* Yamaguti 1952; *Cucullanus* sp.; *Caligus* sp.; and *Aega* sp. (Ruangpan 1982) The parasitic isopod *Aega* sp., appeared to be pathogenic; infected fish lost appetite, became anemic and showed low growth rate. In the Philippines, Velasquez (1975) reported four species of trematodes from the same host: *Lecithochirium neopacificum* Velasquez 1962; *Prosorhynchus luzonicus* Velasquez 1959; *P. celebesensis;* and *Transversotrema laruei* Velasquez 1958.

This study is part of a larger investigation designed to identify the bacterial pathogens and parasites of imported juvenile seabass and those infecting seabass cultured in floating cages in Penang. The results of these bacteriological studies are presented separately.

Materials and Methods

Juvenile seabass were obtained from fish farmers in Penang about 2-3 days after their arrival from Bangkok or Songkla. Fish from Bangkok were shipped by air freight to Penang, whereas those from Songkla were transported by lorry. These imported seabass began to die soon after they were released into the floating cages. Unhealthy juveniles were collected for examination for parasites. Seabass fry grown in floating cages reach a market size of about 500 g in about seven months. Market-size seabass were purchased from fish farmers for the study.

Live fish were examined within 24 hours of collection. The same fish were used for both bacteriological and parasitological examinations. The external surface was examined for gross abnormalities and ectoparasites. Wet mounts of scrapings from the skin and gills were prepared and examined under a compound microscope for protozoans. Fish were then measured and weighed. The gills were removed and each gill filament separated. The gill arches, stomach and intestine were placed in separate petri dishes. The stomach and intestine were split open and scraped into separate filled water beakers, the contents allowed to settle and the supernatant poured off. This procedure was repeated until a clear solution was obtained. The sediment was then examined for parasites under a dissecting microscope.

The kidney, liver and spleen were examined separately by pressing between two glass plates and inspection under a dissecting microscope. The eyes and brain were placed in separate petri dishes, teased apart and examined under a dissecting microscope. The musculature was removed from the vertebral column, sliced thinly and examined for macroscopic cysts or helminths.

Monogeneans, trematodes and cestodes were preserved in 70% alcohol stained in Semichon's carmine and mounted in Canada balsam. Nematodes and copepods were preserved in 2% glycerine in 70% alcohol, cleared by evaporation and examined in glycerine.

Results

Seabass juveniles imported from Thailand were found to be weak on arrival in Penang with darkened body coloration. After a few days in the cages, patches of scales were lost. Fish tended to swim to the surface and often had long whitish fecal wastes.

Seventeen species of parasites were recovered from seabass (Table 1). All three groups of seabass examined showed very high overall prevalence of infection: Songkla, 96.7%; Bangkok, 90.2% and market size, 100%. The overall mean intensities of infection for Songkla and Bangkok were similar, 16.2 and 16.1, respectively. However, the overall mean intensity for market-size fish (115.3) was about seven times that of those from Songkla or Bangkok.

The 17 species of parasites recovered from seabass included two protozoans *(Crytocaryon* sp., *Trichodina* sp.*)*, two monogeneans *(Cycloplectanum latesi* Tripathi 1957; *Cycloplectanum* sp.*)*, six digeneans *(Bucephalus* sp., *Cardicola* sp., *Extenurus* sp., *Lecithochirium neopacificum*, Velasquez 1962, *Pseudometadena celebesensis* Yamaguti 1952, *Rhipidocotyle* sp., one cestode (Tetraphyllidea gen. sp.), two nematodes *(Anisakis* sp., *Raphidascaris* sp.), two isopods *(Aegathoa, Cymothoa* sp.*)*, one brachiuran *(Argulus* sp.*)*, and one copepod *(Caligus* sp.*)*. Only five species of parasites were recovered from the Songkla sample, whereas 12 species were recovered from both the Bangkok and Penang samples. All the parasites from Songkla and Bangkok seabass must have been imported along with their hosts. The most common parasite in both Songkla and Bangkok samples was a diplectanid monogenean, *C. latesi*. The Songkla sample had a prevalence of 91.5% and a mean intensity of 14.6 of this parasite, whereas the Bangkok sample had a prevalence of 78.4% and a mean intensity of 10.8. Two protozoans of known pathogenicity, *Cryptocaryon* sp. and *Trichodina* sp., were also found on the imported fish but were not observed on market-size seabass.

Twelve species of parasites were recovered from market-size seabass, the most common also being *C. latesi*, with a prevalence of 95.7% and a mean intensity of 83.7. However, in the cages, many fish appeared to have acquired large numbers of two digeneans, *P. celebesensis* (prevalence: 87% and mean intensity: 27.1) and *Bucephalus* sp. (39.1% and 11.2). Although *Rhipidocotyle* sp. was found in imported seabass, they seemed to have acquired additional infections of this trematode after being introduced into the cages.

Discussion

As far as can be ascertained from the published literature, the parasite fauna of seabass in its natural habitat has not been well documented. Only Ruangpan (1982) provided information on the prevalence and intensity of parasites of seabass in Thailand. All seabass examined were infected with *Pseudometadena celebesensis*, with an intensity of 9.3, whereas 86% were found infected with *Lecithochirium* sp., with an intensity of 5.5. Furthermore, the acquisition of parasites following introduction of fish into new habitats or the transfer of their parasites to indigenous fish species can then be readily ascertained.

This study contributes to the understanding of the parasite fauna of seabass and its possible role in frequently observed disease outbreaks in juveniles imported for cage culture. The large number of parasites found in imported juveniles from both Bangkok and Songkla should be a concern to Malaysian fish growers, exporters of juveniles and government agencies charged with protecting the aquaculture industry and natural fish stocks. Although fish imported from Bangkok were much smaller than those from Songkla, they were infected with a greater variety of parasites.

The large number of species of parasites and their high intensities in the imported fish may have contributed to the frequent disease outbreaks which occur soon after the fish were released into the cages in Penang. The protozoans *Cryptocaryon* sp. and *Trichodina* sp., found in a large percentage of the imported fish, are known pathogens, especially to fry. Hawke (1976) reported that *Trichodina* sp. and *Chilodonella* sp. caused mortality among striped bass fingerlings when they were held in fiberglass troughs.

The imported juveniles carried high numbers of *Cycloplectanum* spp., particularly *C. latesi*, which should be a concern to fish farmers. Careful examination of moribund or dead fish may show clinical signs attributable to infection by the monogenean. Cultured *Cyprinus carpio* and *Ctenopharyngodon idella* were often killed by monogeneans in China (Anon. 1973). Kabata (1985) reported that a whole population of *Siganus* sp. fry and young fish in experimental culture ponds were killed in Indonesia. Paperna (1963a, 1963b, 1964) found that *Dactylogyrus vastator* causes heavy mortality among carp *(Cyprinus carpio)* fry, whereas *D. extensus* attached mainly to mature carp in Israel. Although there have been few reports that heavy infections of monogeneans cause death of fish per se, injuries caused to the gill filaments by the hamuli and marginal hooklets of their opisthaptors can be considerable, leading to secondary infections which are often fatal.

Another factor which may have contributed to the frequent outbreaks of disease when fish were first released into the cages is stress, especially resulting from transportation. Stressed fishes become more susceptible to infection, especially under the confined environment of the culture cages, where high stocking densities facilitate easy transmission of infective agents.

The present findings indicate that a quarantine system for seabass should be established. Imported fish should be treated at the quarantine station to reduce the risk of introducing imported pathogenic organisms into Malaysian waters. Proper chemical treatment provided at the quarantine station could help lessen the initial mortality in fish cages, thereby reducing the losses to farmers.

The species of parasites recovered from market-size seabass are similar to those recovered from imported juveniles. It is possible that the majority of these parasites was introduced along with the juveniles when the fish were stocked for grow-out in the cages.

The Ciliates *Cryptocaryon* and *Trichodina* were not found in market-size fish. It is possible that as the fish grow in size, they become unsuitable hosts for these protozoans. In contrast, infections by diplectanid monogeneans increase in intensity as the fish grow. This may be due to the fact that monogeneans have a direct life cycle, not requiring an intermediate host, and that the high stocking densities in the cages facilitate their transmission.

Trematodes constituted the largest group of parasites in juveniles from Bangkok and in market-size seabass, but none were recovered from juveniles from Songkla. It appears that the market-size seabass acquired two species of trematodes, *Bucephalus* sp. and *Pseudometadena celebesensis*, while in the cages. Infections could have also been acquired through trash fish fed to the seabass daily.

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Table 1. Prevalence and mean intensity of parasites from seabass, *Lates calcarifer,* imported from Songkla and Bangkok, Thailand and those cultured in Penang, Malaysia.

	Geographical locality					
	Songkla		Bangkok		Penang	
Parasite	Prevalence (%)	Mean intensity	Prevalence (%)	Mean intensity	Prevalence (%)	Mean intensity
Protozoa						
Cryptocaryon sp.	10.6		22.5			
Trichodina sp.			33.3			
Monogenea						
Cycloplectanum latesi	91.5	14.6 ± 15.5	78.4	10.8 ± 11.8	95.7	83.7 ± 83.8
Cycloplectanum sp.	14.9	5.8 ± 3.1	20.6	15.1 ± 12.9	4.4	1.0
Trematoda						
Bucephalus sp.					39.1	11.2 ± 16.3
Cardicola sp.			2.0	2.0 ± 1.0	8.7	1.0
Ectenurus sp.			11.8	1.3 ± 1.5	13.0	3.3 ± 0.9
Pseudometadena celebesensis					87.0	27.1 ± 24.0
Rhipidocotyle sp.			3.9	1.3 ± 0.4	34.8	10.1 ± 8.3
Lecithochirium neopacificum			1.0	5.0		
Cestoda						
Tetraphyllidea gen. sp.	14.9	8.0 ± 5.8	11.8	4.3 ± 4.9	4.4	44.0
Nematoda						
Anisakis sp.			3.9	2.0 ± 0.9	13.0	3.0 ± 2.2
Raphidascaris sp.	2.1	1.0	40.2	4.9 ± 5.8	52.2	1.7 ± 1.3
Brachuira						
Argulus sp.					4.4	1.0
Copepoda						
Caligus sp.					4.4	1.0
Isopoda						
Aegathoa sp.			4.9	1.0		
Cymothoa sp.			1.0	1.0		
No. of fish examined	47.0		102.0		23.0	
Percent of total fish infected	95.7		90.2		100.0	
Mean intensity	16.2		16.1		115.3	
Average length (cm) of fish	10.6 (8.9-12.5)		6.4 (4.8-9.5)		36.8 (27.8-49.5)	
Average weight (g) of fish	13.9 (8.2-20.5)		3.7 (1.5-10.0)		667.4 (360-1,000)	

Parasites of Nile Tilapia *(Oreochromis niloticus)* in the Philippines

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Abstract

From October 1985 to April 1986, detailed parasitological examinations were performed on 175 juvenile and adult Nile tilapia *(Oreochromis niloticus)*. Seventeen different species of parasites (eight Protozoa, five Monogenea, two Digenea, one Copepoda and one Isopoda) were recovered. Parasites commonly encountered during this survey were *Trichodina* spp. *(T. acuta* Lom, 1970; *T. centrostrigata* Basson, Van As, and Paperna, 1983; *T. heterodentata* Duncan, 1973; and *Trichodina* sp.), *Cryptobia branchialis* Nie in Chen, 1955; and *Cichlidogyrus* spp. *(C. sclerosus* Paperna and Thurston, 1969; *C. tilapiae* Paperna, 1960; *C. tiberianus* Paperna, 1960; and *C. longicornis longicornis* Paperna and Thurston, 1969) in freshwater; and *C. branchialis* and *Caligus epidemicus* Hewitt, 1971 in brackishwater collections. Other parasites which were less frequently encountered are Trichodinidae gen. sp., *Apiosoma* sp., *Vorticella* sp., *Gyrodactylus* sp., *digenean metacercariae* and juvenile gnathiid isopods. Although no gross pathology due to parasitic infection was observed, several of the species recovered have been reported to cause diseases in other cultured fishes: A number of the parasites identified are believed to be exotic to the Philippines. Care should thus be taken that they are not introduced into new bodies of water along with Nile tilapia stocked for aquaculture purposes.

Introduction

The Nile tilapia *(Oreochromis niloticus* (L.)) of the family Cichlidae, is a native freshwater fish of South Asia and northern Africa, occurring naturally from Syria southward through eastern Africa to Lake Tanganyika and westward across central Africa to southern Mauritania and Senegal (Philippart and Ruwet 1982). Because of its rapid growth and high consumer acceptability, it is widely distributed for aquaculture and has been introduced into South and Central America, the southern United States, southern India, Sri Lanka, Japan and most of the countries in Southeast Asia. It was introduced into the Philippines on several occasions, the earliest being in 1972 from Israel and Thailand, the original stocks being from Uganda and Egypt, respectively (Guerrero 1985).

In many developing countries, Nile tilapia is a major protein source. In the Philippines, it is an increasingly important food fish, supplanting the less desirable Mossambique tilapia *(Oreochromis mossambicus* (Peters)). In Central Luzon, particularly, market prices for Nile tilapia compare favorably with those of other prominent food fishes, such as milkfish *(Chanos chanos* (Forskal)) (Smith et al. 1985).

Because of their wide geographical distribution, high number of species, and increasing importance to aquaculture, cichlid fishes have recently received increased parasitological attention. Diseases and parasites of wild and cultured cichlids were reviewed by Goldstein (1970, 1973), Sarig (1971), Fryer and Iles (1972), Balarin and Hatton (1979), Roberts and Sommerville (1982) and Paperna et al. (1983). Publications dealing in whole or in part with the parasites of Nile tilapia in countries other than the Philippines include those of Paperna (1960, 1963, 1964, 1965, 1968, 1980); Paperna and Thurston (1969); Ergens (1981); and Kabata (1985), among others. Previous studies recording parasites of Nile tilapia in the Philippines are the reports of Guerrero and Paycana (1981a, 1981b); Quines and Paycana (1982, 1983) and Hopkins and Cruz (1982). To date, no in-depth survey of the parasites of tilapias in the Philippines has been conducted.

The purpose of this investigation is to provide baseline information on the distribution of parasites of Nile tilapia in the Philippines and to evaluate their pathological significance in aquaculture. The results presented herein are from examinations made in the first year of a multi-year study being conducted by the Fish Health Project of the Bureau of Fisheries and Aquatic Resources (BFAR).

Materials and Methods

One hundred juvenile Nile tilapia from Muñoz, Nueva Ecija; and 25 tilapia each from Leganes, Iloilo; Pagbilao, Quezon; and Los Baños, Laguna were examined from October 1985 to April 1986. Fish were examined live on site or transported to the Fish Health Project Laboratory in Quezon City or to the SEAFDEC-AQD laboratory in Tigbauan, Iloilo in oxygenated plastic bags. Details for all host collections, given below, include the collection number, locality, collection date, sample size and host fork length (cm) and weight (g), both expressed as the range followed, in parentheses, by the mean ± one standard deviation.

1. BFAR National Freshwater Fish Hatchery (NFFH) rearing ponds, Muñoz, Nueva Ecija: 10/14/85; n = 25; 7.6-10.9 (8.4 ± 0.7); 12.0-20.0 (15.0 ± 3.0).

2. BFAR NFFH rearing ponds, Muñoz, Nueva Ecija: 2/12/85; n = 25; 5.5-10.8 (8.0 ± 2.1); 2.0-20.0 (11.2 ± 5.2).

3. BFAR NFFH rearing ponds, Muñoz, Nueva Ecija; 29/1/86; n = 25; 12.5-16.0 (13.4 ± 3.3); 32.8-71.0 (51.5 ± 13.0).

4. BFAR NFFH rearing ponds, Muñoz, Nueva Ecija: 2/3/86; n = 25; 4.5-7.3 (6.1 ± 0.6); 1.0-5.0 (3.2 ± 1.2).

5. University of the Philippines in the Visayas, Brackishwater Aquaculture Center culture pond, Leganes, Iloilo: 27/1/86; n = 25; 6.3-12.5 (8.6 ± 3.0); 5.0-40.0 (15.0 ± 10.0).

6. BFAR Brackishwater Aquaculture Demonstration and Training Centre culture pond, Pagbilao, Quezon: 18/2/86; n = 25; 6.8-14.0 (11.4 ± 2.0); 10.0-50.0 (29.1 ± 11.1).

7. Private fish culture pen at Laguna de Bay, Los Baños, Laguna: 21/4/86; n = 25; 4.8-15.0 (9.5 ± 2.0); 1.5-24.7 (12.3 ± 6.0).

Complete host necropsies were performed with the aid of compound and stereomicroscopes using standard parasitological techniques similar to those outlined by Kabata (1985).

Wet mounts of skin and gill scrapings were examined with a compound microscope at 400x magnification for protozoans before placing the fish in 1:4,000 formalin for about 10 min. to relax ectoparasitic helminths and crustaceans. Blood was collected in heparinized capillary tubes after severing the caudal peduncle, and thin smears were prepared and stained with Giemsa's stain prior to examination at 400x and 1,000x magnifications for blood protozoans.

Fish were then killed by a blow on the head and fork length (cm), weight (g) and sex determined. The following tissues and organs were examined: skins, fins, nasal cavities, eyes, opercula, heart, gonads, body cavity, mesenteries, stomach, pyloric caeca, intestines and gills. Visceral organs of small fish were pressed between glass plates and examined with a stereomicroscope. For larger tilapia, the stomach, pyloric caeca and intestines were separated, cut open and their contents individually removed and examined, after which the tissue was pressed between glass plates and examined for larval helminths. Wet mounts were prepared from brain, intestine, kidney, spleen and liver tissue and the gall and urinary bladders and examined at 400x magnification.

The body musculature was removed from the vertebral column and the skin from the flesh. The musculature was then sliced in thin transverse sections, pressed between glass plates and examined for larval helminths and cysts.

Smears of gills infected with *Cryptobia* were fixed in Schaudinn's solution and stained with Heidenhain's iron hematoxylin and mounted in Canada balsam. Photomicrographs and measurements were also made from living flagellates. Klein's dry silver impregnation technique (see Arthur and Lom 1984) was used to identify trichodinids. Living monogeneans were mounted directly in ammonium picrate and examined by phase-contrast optics using the methods of Malmberg (1956). Digeneans were fixed in either hot (70°C) 10% formalin or AFA (alcohol-formalin-acetic acid) and stained with iron hematoxylin or Grenacher's borax carmine. Crustaceans were fixed in 70% ethanol and their appendages mounted in Berlese's fluid.

Results

In this study seventeen species of parasites were recovered from 175 Nile tilapia (Table 1). These include eight protozoans, five monogeneans, two digeneans, one copepod and one isopod.

Four of the protozoans have so far been identified to species: *Trichodina acuta* Lom, 1970; *T. centrostrigata* Basson, Van As and Paperna, 1983; *T. heterodentata* Duncan, 1977; and *Cryptobia branchialis* Nie in Chen, 1955. *Trichodina* spp. were only numerous enough in collections from Muñoz to allow identification to species, where prevalences ranged from 0 to 84%. Included in the Muñoz material was a fourth species of *Trichodina* which was not readily identified. Only a few specimens of *Trichodina* were encountered on individual fish examined from Pagbilao and Los Baños, where prevalences were 48 and 8%, respectively. *Cryptobia branchialis* occurred in all three localities, with a maximum prevalence of 64% being observed at Muñoz. Other Protozoa (Trichodinidae gen. sp., *Apiosoma* and *Vorticella*), encountered only at Pagbilao, were not present in sufficient numbers to allow accurate characterization.

Four species of *Cichlidogyrus* were identified from tilapia from both freshwater localities (Muñoz and Los Baños). These include *C. sclerosis* Paperna and Thurston, 1969; *C. tilapiae* Paperna, 1960; *C. tiberianus* Paperna, 1960 and *C. longicornis longicornis* Paperna and Thurston, 1969. *Cichlidogyrus* spp. were most abundant on tilapia at Los Baños, with a prevalence of 92% and a mean intensity of infection of 22.2 parasites/host. In addition, a few specimens of an as yet unidentified species of *Gyrodactylus* were recovered from Nile tilapia raised in brackishwater culture at Leganes, Iloilo and in freshwater rearing ponds at Muñoz.

The digeneans found during this survey include the brackishwater species *Transversotrema laruei* Velasquez, 1958 found at Pagbilao and small numbers of as yet unidentified metacercariae in both fresh and brackishwater localities (Muñoz and Pagbilao).

One species of parasitic copepod, *Caligus epidemicus* Hewitt, 1971, was abundant on Nile tilapia at both brackishwater localities, reaching a maximum prevalence of 100% and a maximum mean intensity of 111.7 parasites/fish at Leganes. Three juvenile specimens of an as yet unidentified gnathiid isopod were also recovered from two of 25 Nile tilapia from Pagbilao.

Discussion

The results of this preliminary survey show that Nile tilapia cultured in the Philippines have a diverse and abundant parasite fauna. Of the parasites encountered, members of four genera were common in collections from at least one of the localities (prevalences of 30% and above). These include *Trichodina*, *Cryptobia*, *Cichlodogyrus* and *Caligus*. Seven taxa were less frequently encountered (prevalences between 4 and 20%). These include *Apiosoma*, Trichodinidae gen. sp., *Vorticella*, *Gyrodactylus*, *Transversotrema*, digenean metacercariae and gnathiid isopods. The results of additional host examinations from Pagbilao, not given in Table 1, indicate that *Transversotrema laruei* is also a common parasite of Nile tilapia.

None of the ten parasites identified have been previously reported from Nile tilapia in the Philippines. Several of these, however, have been previously recorded from other Philippine fishes. These include *Trichodina acuta*, reported from *Tilapia zillii* and *Oreochromis mossambicus*, and *T. heterodentata*, reported from the same hosts and from *Trichogaster trichopterus*, both by Duncan (1977), *Cichlidogyrus sclerosus*, reported from *O. mossambicus* by Duncan (1973), and *Transversotrema laruei* recorded by Velasquez (1961) from various brackishwater fishes, among them *O. mossambicus*.

Of the remaining parasites so far identified to species, *T. centrostrigata* was described from *O. mossambicus* and other cichlids and cyprinids from South Africa and Israel, but has apparently not yet been reported from *O. niloticus* (Basson et al. 1983). The flagellated gill parasite *Cryptobia branchialis* is a common parasite of cultured and wild cyprinids and other host species in China, the USSR, Europe, Africa and the USA (Ergens and Lom 1970; Paperna 1980; Lom 1980; Shulman 1984). It also has apparently not been previously recorded from Nile tilapia. All species of *Cichlidogyrus* found in this study were previously reported from *Oreochromis niloticus* from Africa (Paperna 1960; Paperna and Thurston 1969). *Caligus epidemicus*, described from various marine fishes of New Zealand (Hewitt 1971) has apparently not been previously reported from cichlid fishes.

Although several species of parasites were occasionally in large numbers (e.g., *Trichodina* spp., *Cichlidogyrus* spp., *C. branchialis* and *C. epidemicus*) gross pathology associated with the presence of any of the species encountered was not observed. As noted by Roberts and Sommerville (1982), tilapias are particularly hardy fishes and do not normally suffer high mortality from parasitic infections. Additional detailed experimental and histopathological studies are needed, however, to assess the pathogenicities of the various parasite species to tilapia and to determine their effects upon the aquaculture industry.

Of additional concern is the presence of a number of apparently exotic parasites *(Trichodina acuta, T. centrostrigata, T. heterodentata* and *Cichlidogyrus* spp.*)* which have entered the country with imported tilapias. Their presence clearly demonstrates the need for effective quarantine and/or certification systems to ensure that fishes entering the Philippines are free from potentially dangerous parasites and diseases.

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Table 1. Parasites of *Oreochromis niloticus* (L.) from freshwater and brackishwater ponds in the Philippines.

Collection no.		1			2			3			4		
			Intensity[2]			Intensity			Intensity			Intensity	
Parasites	Location	Prevalence[1]	Range	$\bar{X} \pm$ SD	Prevalence	Range	$\bar{X} \pm$ SD	Prevalence	Range	$\bar{X} \pm$ SD	Prevalence	Range	$\bar{X} \pm$ SD
Protozoa													
Trichodina spp.	skin, gills				84.0[3]						52.0[3]		
Trichodinidae gen. sp.	skin, gills												
Cryptobia branchialis	gills				32.0						64.0		
Apiosoma sp.	skin, gills												
Vorticella sp.	skin												
Monogenea													
Cichlidogyrus spp.[4]	gills	60.0	2-15	6.8 ± 4.0	60.0			56.0	1-42	7.9 ± 10.6	52.0		
Gyrodactylus sp.	skin	4.0											
Digenea													
Transversotrema laruei	skin												
Digenea gen. sp. metacercariae	muscle, fins							12.0	1-2	1.3 ± 0.6			
Copepoda													
Caligus epidemicus	skin												
Isopoda													
Gnathiidae gen. sp.	skin												

Collection no.		5			6			7		
			Intensity			Intensity			Intensity	
Parasites	Location	Prevalence	Range	$\bar{X} \pm$ SD	Prevalence	Range	$\bar{X} \pm$ SD	Prevalence	Range	$\bar{X} \pm$ SD
Protozoa										
Trichodina spp.	skin, gills				48.0			8.0		
Trichodinidae gen. sp.	skin, gills				20.0					
Cryptobia branchialis	gills				48.0			24.0		
Apiosoma sp.	skin, gills				20.0					
Vorticella sp.	skin				12.0					
Monogenea										
Cichlidogyrus spp.[4]	gills							92.0	1-86	22.2 ± 21.3
Gyrodactylus sp.	skin	8.0	1-2	1.5 ± 0.7						
Digenea										
Transversotrema laruei	skin				4.0					
Digenea gen. sp. metacercariae	muscle, fins				12.0					
Copepoda										
Caligus epidemicus	skin	100	7-548	111.7 ± 116.6	92.0	1-138	34.3 ± 32.2			
Isopoda										
Gnathiidae gen. sp.	skin				8.0	1-2	1.5 ± 0.7			

[1] Prevalence (% infected).

[2] Intensity of infection.

[3] Collections containing *T. acuta*, *T. centrostrigata*, *T. heterodentata* and *Trichodina* sp.

[4] Includes *C. sclerosus*, *C. tilapiae*, *C. tiberianus* and *C. longicornis longicornis*.

Parasites of Juvenile Milkfish, *Chanos chanos*

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Abstract

From September 1985 to April 1986, 200 juvenile milkfish collected from three Philippine localities were examined for protozoan and metazoan parasites and yielded five Protozoa, four Digenea, one Copepoda and one Isopoda. Parasites believed to be previously unreported from *Chanos chanos* include *Cryptobia branchialis* Nie in Chen, 1955; *Apiosoma* sp.; *Ambliphrya* sp.; *Leptotheca* sp.; *Transversotrema laruei* Velasquez, 1958; *Caligus epidemicus* Hewitt, 1971 and an as yet unidentified juvenile gnathiid isopod. Prevalences and intensities of infection for species of parasites encountered were typically low. Only Trichodinidae gen. sp., *T. laruei*, digenean metacercariae and *C. epidemicus* were common in any of the collections.

The potential threat to milkfish culture posed by these parasites and by other species recorded in the literature is discussed. Apart from occasional localized epizootics of ectoparasitic crustaceans, parasites do not appear to be a major problem to the industry.

Introduction

Milkfish is one of the important cultured fishes of Southeast Asia, being raised in large numbers in the Philippines, Indonesia and Taiwan. Milkfish culture in the Philippines may have started as early as the 15th century at Mactan Island in the Visayas (Schmittou et al. 1985). However, Chong et al. (1982) state that the first recorded culture of this species in the Philippines was in 1863 in Rizal Province. At present, there are about 195,000 ha of brackishwater ponds and 30,000 ha of freshwater fishpens devoted to raising milkfish (Marte et al. 1984). Most production is for domestic consumption. In 1984, the total revenue from the export of fry and frozen/chilled milkfish was about US$1.4 million (Bureau of Fisheries and Aquatic Resources 1985).

Although the economic importance of milkfish is well recognized, to date there is only limited information on its parasites and diseases. A review of the literature shows only approximately sixteen different parasites as having been previously reported from milkfish throughout its distribution, the majority of which have not been identified to the species (Table 1).

This study surveys the parasites infecting cultured juvenile milkfish from three localities in the Philippines. Further research, however, is needed to determine the identities of all species and their pathogenicities. This paper contributes to the growing literature on milkfish parasites and can serve as a foundation for further parasitological research on this important species.

Materials and Methods

One hundred and fifty juvenile milkfish were collected from the BFAR Brackishwater Aquaculture Demonstration and Training Center (BADTC) ponds at Pagbilao, Quezon Province, Philippines, from September 1985 to February 1986. Samples of twenty-five juveniles each were also collected from one of the ponds of the BFAR District Fishery Office at Molo, Iloilo Province, and from the Laguna de Bay fishpen of Mr. Menzi Alcantara at Binangonan, Rizal Province, during January and April 1986, respectively. The Pagbilao and Molo collection sites are brackish, with salinities of 2-30 ppt depending on season, while Laguna de Bay is a freshwater lake. Data for all host collections include the following details: collection number, locality, collection date, sample size, and host fork length (cm) and weight (g), expressed as the range followed in parentheses by the mean and standard deviation.

1. BADTC, Pagbilao, Quezon: 17-19/9/85; n = 25; 2.5-16.5 (9.9 ± 4.3); 2.0 - 67.0 (24.5 ± 20.4)

2. BADTC, Pagbilao, Quezon: 22-24/10/85; n = 25; 3.8-12.8 (8.3 ± 2.6); 2.0 - 28.0 (10.1 ± 6.9).

3. BADTC, Pagbilao, Quezon: 27-29/11/85; n = 25; 4.5-21.0 (12.1 ± 4.9); 5.0 - 135.0 (39.9 ± 35.1).

4. BADTC, Pagbilao, Quezon: 16-20/12/85; n = 25; 8.2-16.5 (11.9 ± 2.2); 2.5-8.0 (23.9 ± 18.0).

5. BADTC, Pagbilao, Quezon: 21-23/1/86; n = 25; 8.1-17.5 (13.8 ± 2.4); 5.0-75.0 (39.2 ± 21.1).

6. BADTC, Pagbilao, Quezon: 18-20/2/86; n = 25; 10.6-20.0 (15.5 ± 2.5); 15.0 - 110.0 (56.0 ± 23.6).

7. BFAR District Fishery Office ponds, Molo, Iloilo: 29-31/1/86; n = 25; 9.7-14.8 (11.6 ± 1.4); 11.0 - 35.0 (19.5 ± 7.7).

8. Private fish culture pen, Laguna de Bay at Binangonan, Rizal: 15-18/4/86; n = 25; 11.8-23.0 (19.3 ± 2.4); 95.0 - 175.0 (125.0 ± 23.1).

Most fish were examined fresh on site. A few were preserved in 10% formalin following collection of blood smears and examination of gill and skin scrapings, and transported to the BFAR Fish Health Laboratory in Quezon City for later necropsy. Full examinations were performed on all fish according to the methods of Kabata (1985) with some modifications. The following organs and tissues were examined under a stereomicroscope at magnifications of up to 40x: skin, fins, eyes, mouth, gills, nasal cavities, heart, esophagus, stomach, intestines, liver, spleen, mesenteries, body cavity and musculature. The latter was removed from the vertebral column and pressed between two glass plates for detection of larval helminths and protozoan cysts. The fork length (cm), weight (g) and general condition of the fish were also noted at the time of examination. Wet mounts of skin and gill scrapings, brain, kidney, liver, spleen, intestinal tissues, and urinary and gall bladders were examined under a compound microscope at magnifications of up to 1,000x. Blood was collected using heparinized capillary tubes by severing the caudal artery. Smears were air-dried, stained with Giemsa's stain, and examined at 1,000x magnification.

For identification of trichodinids, gill and skin scrapings of living ciliates were air-dried and stained with 2% $AgNO_3$ (Klein's dry silver impregnation technique). Smears of gill tissue heavily infected with *Cryptobia* were fixed with methanol and stained with Giemsa's stain. Digenea were relaxed and fixed in either hot (70°C) alcohol-formalin-acetic acid (AFA) or 10% formalin, stained with Semichon's acetic-carmine, Heidenhain's iron hematoxylin, or Grenacher's alcoholic borax-carmine and mounted in Canada balsam. Crustaceans were preserved in 70% ethanol and their appendages mounted in Berlese's fluid. Some *Caligus* specimens were digested with 1% KOH overnight prior to mounting in Berlese's fluid.

Results

A total of eleven species of parasites were recovered from 200 juvenile milkfish (Table 2). These include five Protozoa, four Digenea, one Copepoda and one Isopoda. At least six of the species encountered appear to have been previously unreported from *Chanos chanos*: (*Ambliphrya* sp.; *Cryptobia branchialis* Nie in Chen, 1955; *Leptotheca* sp.; *Transversotrema laruei* Velasquez, 1958; *Caligus epidemicus* Hewitt, 1971 and gnathiid isopods). A seventh species, *Apiosoma* sp., also not previously recorded from milkfish, was encountered in this study at Pagbilao in samples not listed in this paper.

Several of the parasites could not be assigned to lower taxa because they were either not recovered in sufficient numbers *(Trichodina, Ambliphrya, Scyphidia* and *Leptotheca)* or were immature (digenean metacercariae, Gnathiidae gen. sp.). Three species of metacercariae from the musculature and fins of milkfish were also recovered. Further studies through feeding experiments will be conducted to establish their identity.

Discussion

Our preliminary findings show that juvenile milkfish are host to a large number of parasite species. However, the prevalences and intensities of infection of these parasites are generally quite low. Only four parasites (Trichodinidae gen. sp., *Transversotrema laruei*, digenean metacercariae and *Caligus epidemicus)* were found with prevalences greater than 30% in any of the collections. Similarly, intensities of infection were likewise extremely low, reaching maximum mean values of only 5.2 for *T. laruei*, 5.2 for digenean metacercariae and 8.8 for *C. epidemicus*.

It is noteworthy that the parasite fauna of young milkfish in culture is dominated by ectoparasitic species, only four of the eleven parasites encountered (three digenean metacercariae and the myxosporean *Leptotheca* sp.) being endoparasitic. Remarkably, no helminths were recovered from the gastrointestinal tract of any of the fish examined. The rapid fluctuations in salinity observed in brackishwater ponds in the Philippines due to frequent heavy rainfall may play a major role in controlling the ectoparasites of cultured milkfish.

No evidence of gross pathology due to parasitic infection was observed in any of the milkfish examined. However, a number of the parasites identified in this survey or recorded by previous workers are known to occasionally cause problems in fish culture. Although *Trichodina* and *Trichodinella* have not been reported to cause problems in milkfish culture, they are occasionally responsible for mass mortalities of other cultured fishes, especially during the hot months (Kabata 1985). Heavily-infected fish may exhibit abnormal behavior and coloration, lose weight and eventually become sluggish and moribund. Chen (1955) reported that *Cryptobia branchialis* was a serious pathogen of cultured *Mylopharyngodon aethiops* in China, destroying gill epithelium and causing high host mortalities. In contrast, Lom (1980), using scanning electronmicroscopy, suggested that *C. branchialis* is an ectocommensal and is not directly pathogenic to fish. Various species of metacercariae are reported to cause mortality, irritation and stress of fish during the first stages of infection (Kabata 1985). Additionally, at least three of the species of metacercariae occurring in Philippine milkfish, *Procerovum varium* Onji and Nishio, 1916; *P. calderoni* (Africa and Garcia, 1935); and *Haplorchus okogawai* (Katsuta, 1932) can develop into adults in man (Velasquez 1979). A species of *Caligus, C. patulus* Wilson, 1937, not encountered during this survey, has been linked to mortalities of milkfish held in experimental tanks in the Philippines (Lavina 1977, 1978). Another parasitic copepod, *Lernaea cyprinacea* Linnaeus, 1758, is a well known pathogen of freshwater fishes in Southeast Asia (Kabata 1985). Pathogenicity severe enough to cause direct mortalities has been observed in young milkfish from Laguna de Bay (Arthur, unpublished data). Infections by the branchiuran *Argulus* have been reported to cause weight loss and mortalities of *C. chanos* in overwintering ponds in Taiwan (Bardach et al. 1972). The parasitic isopods *Alitropus typus* Edwards, 1840 and *Ichthyoxenus* sp., also not encountered in this study, have been reported to cause serious kills of cultured Philippine milkfish (Velasquez 1979). Ronquillo and Caces-Borja (1960) also reported losses of young cultured milkfish due to an unidentified cymothoid isopod.

It can be concluded that parasites are not usually a direct cause of major kills of cultured milkfish. However, parasitism may have less obvious effects on cultured fish. Infections may lead to continuous but less dramatic mortalities, impair growth and cause indirect losses by opening up avenues for secondary invasion by microbes. Thus, more intensive research will be required to fully understand the role parasites play in limiting milkfish production.

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Table 1. Summary of the parasites reported from milkfish.[1]

Parasite	Location	Locality	Reference
Protozoa			
Amblishrya sp.	skin	Philippines	Orig.[2]
Apiosoma sp.	gills	Philippines	Orig.
Cryptobia branchialis	gills	Philippines	Orig.
Cryptobia sp.	gills	Philippines	Kabata 1985
Dinoflagellida gen. sp.	gills	Philippines	Anon. 1973b
Leptotheca sp.	kidney	Philippines	Orig.
Scyphidia sp.	gills	Philippines	Anon. 1973b; Orig.
Sessilina gen. sp.[3]	skin	Philippines	Anon. 1973b
Trichodina sp.	skin, gills	Philippines	Anon. 1973b; Orig.
Trichodinella sp.	gills	Philippines	Kabata 1985
Monogenea			
Monogenea gen. sp.	skin	Philippines	Anon. 1973a, 1973b
Digenea			
Digenea gen. sp. (m)[4]	muscle, fins	Philippines	Anon. 1973b; Orig.
Haplorchus yokogawai (m)	muscle	Philippines	Velasquez 1979
Kruusdeitrema chanosi	intestine	India	Zhukov 1972
Procerovum calderoni (m)	muscle	Philippines	Velasquez 1973, 1975a
P. varium (m)	muscle	Philippines	Velasquez 1973, 1975a
Transversotrema laruei	skin	Philippines	Orig.
Acanthocephala			
Acanthocephalus sp.	intestine	Philippines	Velasquez 1979
Acanthocephala gen. sp.	intestine	Philippines	Velasquez 1975b
Copepoda			
Caligus epidemicus	skin	Philippines	Orig.
C. patulus Syn.: *Caligus* sp. auctorum	skin	Philippines, Philippines Philippines Indonesia	Laviña 1977, 1978; Velasquez 1979; Jones 1980; Kabata 1985
Caligus sp.	(skin)	Indonesia	Jones 1980
Lernaea cyprinacea	skin	Philippines	Velasquez 1979; Orig.
Branchiura			
Argulus sp.	(skin)	Taiwan	Bardach et al. 1972
Isopoda			
Alitropus typicus	skin	Philippines, Indonesia	Velasquez 1979; Kabata 1985
Cymothoinea gen. sp.	skin	Philippines	Ronquillo and Caces-Borja 1960
Gnathiidae gen. sp.	skin	Philippines	Orig.
Ichthyoxenous sp.	skin	Philippines	Velasquez 1979
Isopoda gen. sp.	skin	Philippines	Anon. 1973b

[1] Not listed in this table are the reports of unidentified flatworms and crustaceans from the skin of milkfish by Rabanal et al. (1961).

[2] Orig. = original record.

[3] Reported as an unidentified stalked protozoa by Anon. (1973b).

[4] m = metacercarial stage.

Table 2. Parasites collected from juvenile milkfish *(Chanos chanos)*.

Collection no.[1]	1			2			3			4		
		Intensity			Intensity			Intensity			Intensity	
Parasites	Prevalence[2]	Range	$\bar{X}$ ± SD	Prevalence	Range	$\bar{X}$ ± SD	Prevalence	Range	$\bar{X}$ ± SD	Prevalence	Range	$\bar{X}$ ± SD
Protozoa												
Cryptobia branchialis				12.0								
Leptotheca sp.				8.0								
Scyphidia sp.				20.0								
Trichodina sp.				8.0								
Trichodinidae gen. sp.	60.0			44.0			8.0			8.0		
Digenea												
Transversotrema laruei	8.0	1	1.0 ± 0.0	40.0	1-16	6.2 ± 6.2	32.0	1-11	3.5 ± 3.4	76.0	1-15	3.4 ± 3.8
Digenea gen. sp. Metacercariae	8.0	1	1.0 ± 0.0	16.0	1-4	1.6 ± 1.5	8.0	1-3	2.0 ± 1.4			
Copepoda												
Caligus epidemicus							16.0	1-6	3.2 ± 2.2	4.0	1	1.0 ± 0.0
Isopoda												
Gnathiidae gen. sp.							4.0	1	1.0 ± 0.0			

Collection no.[1]	5			6			7			8		
		Intensity			Intensity			Intensity			Intensity	
Parasites	Prevalence	Range	$\bar{X}$ ± SD	Prevalence	Range	$\bar{X}$ ± SD	Prevalence	Range	$\bar{X}$ ± SD	Prevalence	Range	$\bar{X}$ ± SD
Protozoa												
Cryptobia branchialis				20.0						4.0		
Leptotheca sp.												
Scyphidia sp.				4.0								
Trichodina sp.										4.0		
Trichodinidae gen. sp.				24.0						12.0		
Digenea												
Transversotrema laruei	52.0	1-9	2.9 ± 2.5	12.0	1	1.0 ± 0.0						
Digenea gen. sp. Metacercariae	80.0	1-9	4.0 ± 3.0	44.0	1-18	6.2 ± 6.0						
Copepoda												
Caligus epidemicus	12.0	1	1.0 ± 0.0	36.0	1-40	8.8 ± 13.2	4.0	1	1.0 ± 0.0			
Isopoda												
Gnathiidae gen. sp.												

[1] For localities see Materials and Methods.

[2] Expressed as % infected.

The Reproductive Biology of *Dactylogyrus nobilis* (Monogenea: Dactylogyridae) from the Gills of Bighead Carp *(Aristichthys nobilis)*

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Abstract

Dactylogyrus nobilis, a monogenean found on the gill filaments of bighead carp *(Aristichthys nobilis)*, has oval eggs with a polar filament at one end. The eggs measure 54.34 (48.00-68.00)µm long by 33.71 (30.00-40.00)µm wide, increasing in size during embryonic development (three to four days). On the fourth day hatching occurs. Egg development and hatching are favored in the presence of host mucus. The egg production of *D. nobilis* on excised gill arches at 32°C is 1.14 eggs per worm. In experiments where the fish are kept in compartmentalized tanks measuring 17 x 15 x 15 cm at 27°C, the egg production of *D. nobilis* is 2.08 eggs per worm.

Introduction

The reproductive biology of the Monogenea is known mainly from a few individuals of the *Dactylogyrus* and *Gyrodactylus* genera (Iziumova 1956; Paperna 1963a, 1963b; Prost 1963; Khalil 1964; Molnar 1971; Lester and Adams 1974a, 1974b; Imada et al. 1976). One of the most extensive of such studies on *Dactylogyrus* is that of Prost (1963) who investigated the development and pathogenicity of *Dactylogyrus anchoratus* and *Dactylogyrus extensus* for breeding carps. Among her findings of significance for the management of infections in ponds were that dactylogyrids were susceptible to freezing and drying and that there was a strict correlation between the number of eggs laid and temperature.

The effect of temperature on the oviposition of Monogenea was studied by Iziumova (1956) and Prost (1963). Iziumova (1956) showed that *D. vastator* normally deposited from 4 to 10 eggs in 24 hours but under unfavorable conditions or at a higher temperature the number of eggs deposited increased. This was similar to the findings of Imada and Muroga (1978) who found that *Pseudodactylogyrus microchis* on cultured eels increased their oviposition rate initially but it then fell to 7.7 (3.0-9.8) at 28°C. There is still a huge gap in the current knowledge of the biology of other Monogenea from warmwater fish. It is known that egg production of Monogenea increases when the environment is adverse (Iziumova 1958). Hence high temperature, low oxygen content and even death of hosts will lead to greater egg deposition.

Bauer et al. (1973) have given the bionomy of *D. aristichthys* and *D. nobilis*. Lucky (1973) only found and described specimens of *D. nobilis* in ponds in Czechoslavakia. To date there has been no work done on the Monogenea of bighead carp in the tropics. Shaharom (in press) studied the Monogenea of bighead carp from cultured ponds in Malaysia and found *D. nobilis* the prevalent parasite at most times of the year. This study is therefore to obtain further details on some aspects of the reproductive biology of *D. nobilis*.

Materials and Methods

The fish were obtained from two Malaysian importers: Ban Lee, Salak South, Selangor and South-East Asian Farm, Enggor, Perak, and were kept in large circular fibron tanks.

The eggs of the Monogenea were obtained from worms laying eggs on freshly excised gills. Observations were done under a dissecting microscope and eggs were pipetted out and placed in embryo dishes, together with some aquarium water in which the host fish (bighead carp, *Aristichthys nobilis)* had been kept. The eggs were placed in batches of 10 in cavity blocks containing sterilized aquarium water. The cavity blocks were kept at ordinary room temperature and were examined daily using a stereo dissecting microscope. One egg was taken out daily, placed in a drop of aquarium water on a glass slide, and a coverslip placed on it. It was examined under 10x and 40x magnification using an Olympus microscope. Eggs of *D. nobilis* were also placed in different media such as distilled water, distilled water plus host mucus as well as sterilized aquarium water.

In a second series of experiments, fish were kept in aerated compartmentalized glass tanks measuring 17 x 15 x 15 cm. Each compartment contained one fish. After an interval of one day each fish was killed and the gills examined for Monogenea. The water from each compartment was filtered using filter paper and a vacuum pump. Eggs were counted from the filter paper under a 20x magnification. The fecundity of the worms under natural conditions could therefore be elucidated. Eggs laid by Monogenea from excised gill arches were also determined. These were done by cutting each gill arch, placing it in a cavity block with some aquarium water and leaving for two hours. At the end of this period the number of eggs laid by each worm was counted.

Results

The eggs of *D. nobilis* were oval with a short polar filament at one end. They measured 54.34 (48-68)μm long and 33.71 (30.00-40.00) μm wide. The embryonic development of the eggs of D. nobilis was observed (Fig. 1).

On the first day of incubation the newly laid oval egg was 60μm long and 40μm wide. Inside the egg were dense granules. On the second day of incubation the eggs grew slightly to 62μm long and 48μm wide. The dense granules appeared to align themselves in specific positions. The two black eyespots could be seen. The eggs incresed to 82μm long and 54μm wide on the third day of incubation. The embryo inside was almost completely formed with four black pigmented eyespots and marginal hooks. On the fourth day of incubation the eggs grew to 92μm and 54μm width. The eyespots and marginal hooks were fully formed. The embryo moved quickly and the cilia beat rapidly back and forth. At this point it was almost ready to hatch. At hatching, the operculum or lid opposite the polar filament was pushed open, the head of the embryo thrust out and the oncomiracidium swam away. At hatching, the oncomiracidium was 186μm long and 90μm wide. When newly hatched, the oncomiracidium swam quickly in a spiralling movement upwards every 20 seconds. Its movements then slowed down but when the water was shaken it swam upward again.

The incubation period for several batches of eggs kept in different media is shown in Table 1. Eggs incubated in distilled water showed no development even after three days. Eggs incubated in distilled water with host mucus gave 68% and 73% hatching. Eggs incubated in sterilized aquarium water gave 6.6% hatching after two days and 43.3% hatching ater four days.

The egg production of *D. nobilis* on excised gill arches at 32°C is shown in Table 2. Mean egg production was 1.14 eggs/worm.

The average fecundity was 0.58. Fecundity is expressed as the ratio of eggs laid per worm in relation to a time period.

Table 3 shows the egg production of *D. nobilis* from gills of *Aristichthys nobilis* kept in glass compartmentalized tanks with water temperature of 27°C.

Discussion

The embryonic development of the egg *D. nobilis* is similar to that of other dactylogyrid eggs (Iziumova 1958; Paperna 1963a, 1963b; Prost 1963) and also ancyrocephalid eggs of *Urocleidus adspectus* (Cone 1979) and *C. sclerosus* (Shaharom 1983). The swelling of the egg of *D. nobilis* during incubation resembled *C. sclerosus* (Shaharom 1983) and *D. vastator* (Paperna 1963b). The viscous cushion seen in the eggs of *C. sclerosus* (Shaharom 1983) was not present in the eggs of *D. nobilis*. The swelling of the viscous cushion leads to expansion within the egg (Wilson 1968) and although *D. nobilis* does not have the viscous cushion it still expands during embryonic development.

Eggs placed in distilled water without host mucus did not develop while eggs exposed to distilled water and host mucus developed and hatched. According to Macdonald (1974), the host mucus dissolves the opercular cement or activate the larva, which in turn secretes a hatching enzyme capable of softening the opercular cement and causing the larva to extend its body and push out the operculum.

The behavior of the oncomiracidiuim within the egg is similar to that described by Cone (1979) and Shaharom (1983). The oncomiracidium expanded and contracted its body with the anterior end thrusting towards the operculum. Many authors have reported mechanical dislodgement of the operculum by thrusting movements of the oncomiracidium (Frankland 1955; Bychowsky 1961; Bovet 1967; Molnar 1971; Macdonald 1974).

Ljaiman (1951) showed that *D. vastator* laid 5.17 eggs/hour at 28°C. He measured the egg production rate of parasites removed completely from the gills under artificial conditions. Paperna (1963b) obtained an oviposition rate for *D. vastator* of 1.68/hour at room temperature of 28°C. Paperna carried out his experiments under natural conditions, that is, from the gills of live fish kept in small beakers.

The fecundity of 3.79 in Table 3 was obtained because only one Monogenea was found which appeared to have laid 91 eggs. But there is a strong possibility that some of the Monogenea present could have died. The number of eggs laid by *D. vastator* depends on temperature and on the age of the worm (Ljaiman 1951). *D. vastator* eight to nine days old produce from one to three eggs per day; worms 11 to 13 days old produce 5-15 eggs. After that age, the egg deposition gradually diminishes but there is one strong increase just prior to the death of the worm.

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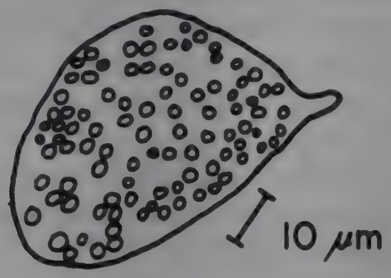
Table 1. Incubation period of eggs of *D. nobilis* in different media.

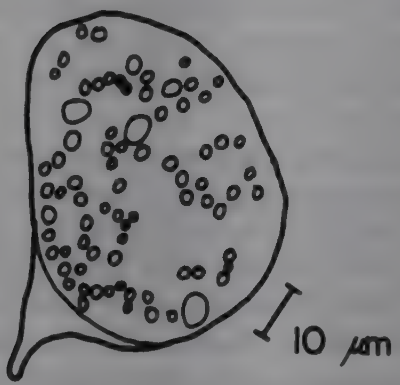
Date incubated	Date examined	Interval in days	No. of eggs	Medium	No. of eggs developed	No. hatched	Host mucus added	% developed	% hatched
6/4/84	8/4/84	2	25	distilled water	nil	nil	nil	0	0
6/4/84	9/4/84	3	25	distilled water	nil	nil	nil	0	0
6/4/84	11/4/84	5	25	distilled water and host mucus	not recorded	17	yes	–	68
6/4/84	11/4/84	5	15	distilled water and host mucus	not recorded	11	yes	–	73
7/4/84	9/4/84	2	60	sterilized aquarium water	26	4	nil	43.3	6.6
7/4/84	11/4/84	4	60	sterilized aquarium water	22	26	nil	3.66	43.3

Table 2. The egg production of *Dactylogyrus nobilis* from excised gill arches of *Aristichthys nobilis*.

Fish species	Length (cm)	Temperature °C	Time interval in hours	No. of worms	No. of eggs	Mean egg/worm	Fecundity
A. nobilis	7.7	32	2	8	8	1	0.5
A. nobilis	7.7	32	2	6	9	1.5	0.75
A. nobilis	7.5	32	2	8	8	1	0.5
		Total	6	22	25		



Ist day of incubation



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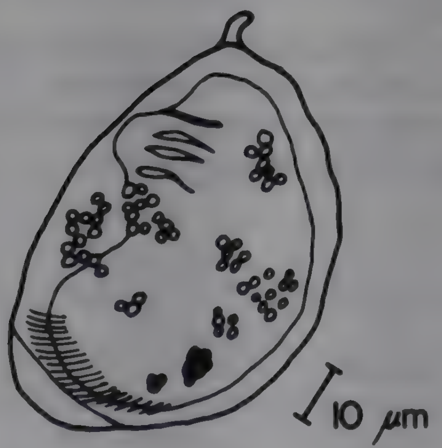
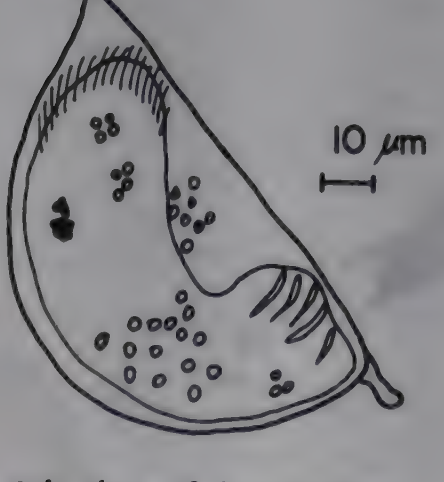


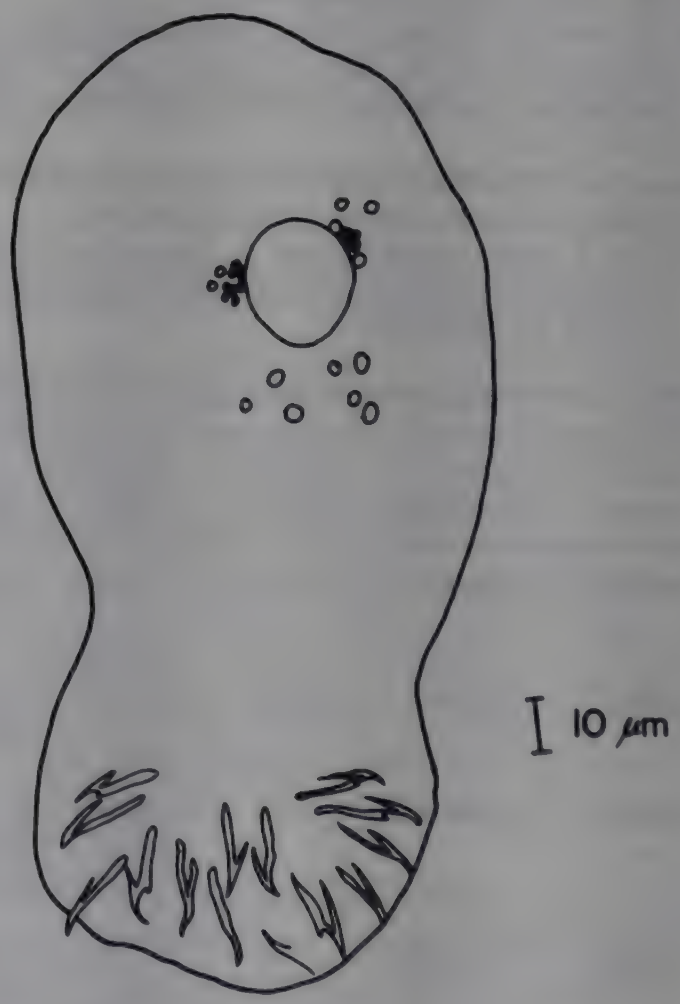
3rd day of incubation



4th day of incubation

Table 3. Egg production of *D. nobilis* from gills of *A. nobilis* kept in experimental tanks with a water temperature of 27°C, at 1-day interval.

Length fish (cm)	No. of worms	No. of eggs	Vol. of water	Mean egg/worm	Fecundity
8.7	0	0	–	0	0
7.7	1	91	1,570	91	3.79
9.5	0	0	–	0	0
9.5	2	17	1,970	8.5	0.35
9.7	0	0	–	0	0
9.6	0	0	–	0	0
9.5	0	0	–	0	0
8.0	7	9	1,370	1.29	0.05
7.7	0	0	–	0	0
7.5	3	5	1,590	1.67	0.07
7.5	6	8	1,600	1.33	0.06
9.5	3	7	1,670	2.33	0.10
8.0	7	9	–	1.29	0.05
8.5	57	80	1,105	1.4	0.06
8.2	42	65	1,250	1.55	0.06
7.5	26	7	–	0.26	0.01
7.5	60	155	1,080	2.58	0.11
			Average	2.08	0.28 ± 0.91



Oncomiracidium (hatching occurs on 4th day)

Fig. 1. The egg development of *Dactylogyrus nobilis* (Monogenea) on the gills of bighead carp *(Aristichthys nobilis)*.

Identification and Distribution of *Lernaea* spp. in Peninsular Malaysia

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Abstract

This survey on the identification and distribution of *Lernaea* spp. in Peninsular Malaysia involved the leading importers of fish fry, all the eight government breeding stations and 104 private ponds selected from all geographic areas of the Peninsula. The study revealed that *Lernaea* infection was present in all the eight government-owned fish breeding stations, in 69.2% of the 104 private fishponds, and in four out of five consignments of fingerlings imported from Taiwan through the importing agency. The source of *Lernaea* at the private farms was traced to the government breeding stations and the importing agency. *Lernaea cyprinacea* "Asian" form which is more widely distributed and *L. polymorpha* were the species present in the ponds. *L. cyprinacea* was found to be widespread at a wide range of temperature (17-32°C) and pH of 5.6-9.0. *L. polymorpha* infected only *Aristichthys nobilis* whereas *L. cyprinacea* infected a wide range of hosts. *Trichogaster pectoralis* and *Tilapia* spp. were not susceptible to infection. The effect of the widespread distribution of *Lernaea* on the aquaculture industry is discussed.

Introduction

The presence of *Lernaea* spp. in Peninsular Malaysia was first recorded in 1960 at the Freshwater Fisheries Research Institute, Malacca (Anon. 1960). The occurrence of *L. piscinae* (=*L. polymorpha* according to Shariff 1985) was more recently reported by Shariff (1981) from the Universiti Pertanian ponds. The presence of *Lernaea* spp. has also been noted in many of the private fish farms (Shariff, unpublished data). *Lernaea* has been reported to be responsible for serious losses to the aquaculture industry (Hoffman 1976; Kabata 1979; Djajadiredja et al. 1983). This study was thus conducted to survey the distribution of the parasite in Peninsular Malaysia and to identify the different species present. An attempt was made to trace the source of the infection and to assess the effects on the local aquaculture industry.

Materials and Methods

The survey of the occurrence and species distribution of *Lernaea* spp. involved the whole of Peninsular Malaysia and covered three categories, namely: (1) private ponds belonging to individual farmers; (2) ponds at the government breeding stations and (3) an importing agency which was centrally located and also the main importing agency in Malaysia. The farmers obtained their supply of fingerlings from both the importing agency and the government breeding stations.

One hundred and four out of the 13,852 private fishponds from 10 different states were chosen for this study. All areas with aquaculture activities were represented, and ponds were selected on a random basis from each area. *Aristichthys nobilis* of 5-10 cm standard length were used to detect the presence of *Lernaea* in the fishponds. Fish that were free of *Lernaea* infection were treated with formalin at 166 ppm for 30 minutes as a prophylactic measure.

In each of the selected ponds, 10 test fish were released into cages and were left in the ponds for a minimum of four days. After four days the fish were removed and examined for the presence of adult *Lernaea* spp. The parasites were then removed by dissection and identified to species level based on the key prepared by Harding (1950).

A. nobilis from five different consignments were examined from an importing agency in Kuala Lumpur. The fish were imported from Taiwan, and the five different consignments arrived over a period of four months, April-August 1982. On the arrival of each consignment, 50 live fish were transported to the laboratory where they were kept in aquarium tanks fitted with undergravel filters. The fish were examined for the adult parasite after being kept in the aquarium for two weeks. The longer duration was a precaution taken to allow for the possibility that the imported fish may have been infected with early larval stages of the parasite which would require a further two weeks to reach maturity.

The study involved all the eight government fish breeding stations in Peninsular Malaysia. Attempts to determine presence or absence of the parasite were made using the following sampling methods: (1) introduction of 10 caged *A. nobilis* as test fish into three ponds as described above and (2) examination of five broodstock and 20 fry of each species which were already present in the ponds at the station. The presence or absence of the parasite was recorded if it occurred on any one or all of the three samples.

The water temperatures were recorded by a minimum and maximum thermometer and the pH was measured with the HACH kit.

Results

Fig. 1 shows the 113 locations for the presence or absence of the parasite. The results of the survey revealed that *Lernaea* was present in all the 10 states where the study was conducted. Seventy-two of the 104 private ponds studied (69.2%) revealed the presence of *Lernaea* (Table 1). *L. polymorpha* (Fig. 2) was found in only five samples (4.8%) and was always present together with *L. cyprinacea* "Asian" form (Fig. 3). The remaining samples were identified as *L. cyprinacea* "Asian" form.

Adult parasites were not present on the newly imported fish but by the 14th day parasites were seen on the fish. *Lernaea* was present in four out of the five consignments (Table 2). The species identified were *L. cyprinacea* (14.8%) and *L. polymorpha* (39.6%). The mean intensity of infection was also higher for *L. polymorpha* which was 1.81 parasites as compared to 1.47 for *L. cyprinacea.*

Lernaea was present in all the breeding stations studied. Infection by *L. cyprinacea* "Asian" form was present in several host species (Table 3); and *L. polymorpha* was recorded only from *A. nobilis* at two stations. *Lernaea* spp. was not found on tilapia species or *Trichogaster pectoralis.*

The maximum and minimum diurnal water temperatures recorded from all the ponds studied were 23-32oC, but lower temperatures of 17-24oC were recorded from one breeding station which was sited on a hill. The pH of water ranged from 5.4 to 9.0. *L. cyprinacea* and *L. polymorpha* were present in both extremes of pH (5.4-9.0) and in water temperatures of 23-32oC.

Discussion

There were no quarantine procedures to examine and ensure that the new stocks introduced into the breeding stations were free of the disease nor were there any quarantine procedures for imported fish. Similarly there were no measures for the examination and treatment of fish before they were supplied to the fish farmers. Thus, the free flow of infected fish from the breeding stations and the importing agency resulted in the wide spread of *Lernaea* in Peninsular Malaysia. Although the study involved only one importing agency, there were more than 20 other known agencies which imported fish from the Far East. Besides *A. nobilis* and *C. idella* which are cultured for food, various species of ornamental fish are also being imported. There is evidence that *Lernaea* is brought into the country along with imported ornamental fish (Shariff 1980). Many fish dealers handle both food fish and ornamental fish among which the spread of *Lernaea* could be aggravated.

The importing agency disclosed that fish infected with adult parasites were culled before being sold. The agent was ignorant of the fact that the fish he sold were infected with the larval stages that were not visible to the naked eye.

The finding that *L. cyprinacea* "Asian" form and *L. polymorpha* were present in a wide range of pH (5.6-9.0) is considered to be significant since Hoffman (1976) had reported that *Lernaea* has not been recorded from waters with pH lower than 7. *Lernaea* was also present at a wide range of water temperatures (17-33oC). According to Shields and Tidd (1968) the most successful laboratory cultures of *L. cyprinacea* were obtained at fluctuating temperatures of 24-29oC which are closely similar to the normal diurnal temperatures in Malaysia. Thus, the tropical climate of Malaysia which experiences no seasonal temperature changes could be considered favorable for the development of *Lernaea* throughout the year.

Host specificity could be one of the reasons for the lower number of private fishponds (4.8%) found to be infected with *L. polymorpha* in contrast to *L. cyprinacea* which was found to be more common (69.2%) and had a wide range of host susceptibility. The wide range of host susceptibility of *L. cyprinacea* has also been reported by Hoffman (1967), Kabata (1979) and Shariff et al. (1986). The widespread occurrence of *L. cyprinacea* is also attributed to its ability to live on many unrelated fish hosts such as tadpoles, adult frogs and salamander; thus, *L. cyprinacea* has been recorded to occur in Europe, Africa, India, Southeast Asia, Far East Asia, North and South America (Kabata 1979). Besides the broad range of host susceptibility of *L. cyprinacea,* the wide range of pH and temperature tolerance are probably important factors that have enabled the parasite to be distributed worldwide.

L. polymorpha, which was noted to be host specific, has been previously recorded in China on *A. nobilis* and *Hypophthalmichthys molitrix* (Yin et al. 1963). Kabata (1979) in his review of the geographic distribution of *L. piscinae* Harding (=*L. polymorpha*) has referred to it as an African form whereas the specimens of *L. polymorpha* were actually obtained by Harding from Singapore situated in Southeast Asia. The distribution of *L. polymorpha* therefore still appears to be limited to the Far East and Southeast Asia.

Mortality rates ranging from 42 to 100% due to lernaeosis have been recorded at the importing agency (Ban Lee, pers. comm.). The average mortality rates due to lernaeosis at the breeding stations for 1981 and 1982 have been reported to be 55.6% (Shariff and Vijiarungam 1983). However, at the private farms the effects of the disease may not be so obvious. Most of the private farms practice aquaculture on extensive scale and in these cases the management is at a lower level than in the intensive systems; thus records are not kept well enough to assess the mortality rates.

Factors such as weight loss or slow growth rates caused by lernaeosis could reduce the income of the fish farmer. Even with low *Lernaea* infection, signs of stress reaction indicated by an increase in oxygen consumption have been reported by Srinivasachar and Shakuntala (1975); thus, even under natural conditions, low oxygen levels could be detrimental to the fish. The study on growth rates of *A. nobilis* infected with *L. polymorpha* indicated a difference of 35% in the mean weight when compared to be uninfected controls at the end of the six-month period (Shariff 1985). Besides such effects, there is also the danger that the parasite may spread to the natural water bodies on a large scale due to the further development of aquaculture, and the disease may become a threat to the natural fish population.

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Table 1. Results of the survey of the identification and distribution of *Lernaea* spp. from private farms in West Malaysia.

States	No. of ponds studied	No. of ponds with *L. piscinae*	No. of ponds with *L. cyprinacea*	No infection
Johore	12	0	9	3
Kedah	8	0	6	2
Kelantan	13	0	7	6
Malacca	5	1	3	2
Negeri Sembilan	10	0	7	3
Pahang	17	1	13	4
Perak	16	2	12	4
Selangor	15	1	10	5
Trengganu	8	0	5	3
Total	104	5	72	32

Table 2. Prevalence of *L. cyprinacea* "Asian" form and *L. piscinae* on *A. nobilis* obtained from the importing agency.

Consignment no.	No. of fish examined	Prevalence (%) of		Mean intensity	
		L. polymorpha	*L. cyprinacea*	*L. polymorpha*	*L. cyprinacea*
1	50	52	16	1.68	1.37
2	50	28	14	2.00	2.50
3	50	60	4	1.60	1.00
4	50	58	40	3.78	2.50
5	50	0	0	0.00	0.00
Total	250	$\bar{x}$ 39.6	$\bar{x}$ 14.8	$\bar{x}$ 1.81	$\bar{x}$ 1.47

Table 3. Results of the examination of various host species for *Lernaea* spp. at the government breeding stations in Peninsular Malaysia.

Station (State)	Host spp.	*Lernaea* spp.
Enggor (Perak)	*C. carpio*	*L. cyprinacea* "Asian" form
	T. pectoralis	–
	Tilapia sp.	–
	P. gonionotus	*L. cyprinacea* "Asian" form
Tapah (Perak)	*C. carpio*	*L. cyprinacea* "Asian" form
	P. gonionotus	*L. cyprinacea* "Asian" form
	H. temmincki	*L. cyprinacea* "Asian" form
	O. gouramy	*L. cyprinacea* "Asian" form
Tanah Rata (Pahang)	*C. carpio*	*L. cyprinacea* "Asian" form
Bukit Tinggi (Pahang)	*A. nobilis*	i) *L. cyprinacea* "Asian" form ii) *L. polymorpha*
	P. gonionotus	*L. cyprinacea* "Asian" form
Jitra (Kedah)	*C. carpio*	*L. cyprinacea* "Asian" form
	P. gonionotus	*L. cyprinacea* "Asian" form
Kong-Kong (Johore)	*C. carpio*	*L. cyprinacea* "Asian" form
Batu Berendam (Malacca)	*A. nobilis*	i) *L. cyprinacea* "Asian" form ii) *L. polymorpha*
	P. gonionotus	*L. cyprinacea* "Asian" form
	C. carpio	*L. cyprinacea* "Asian" form
	C. idella	*L. cyprinacea* "Asian" form
Machang (Kelantan)	*C. carpio*	*L. cyprinacea* "Asian" form
	C. idella	*L. cyprinacea* "Asian" form
	P. gonionotus	*L. cyprinacea* "Asian" form

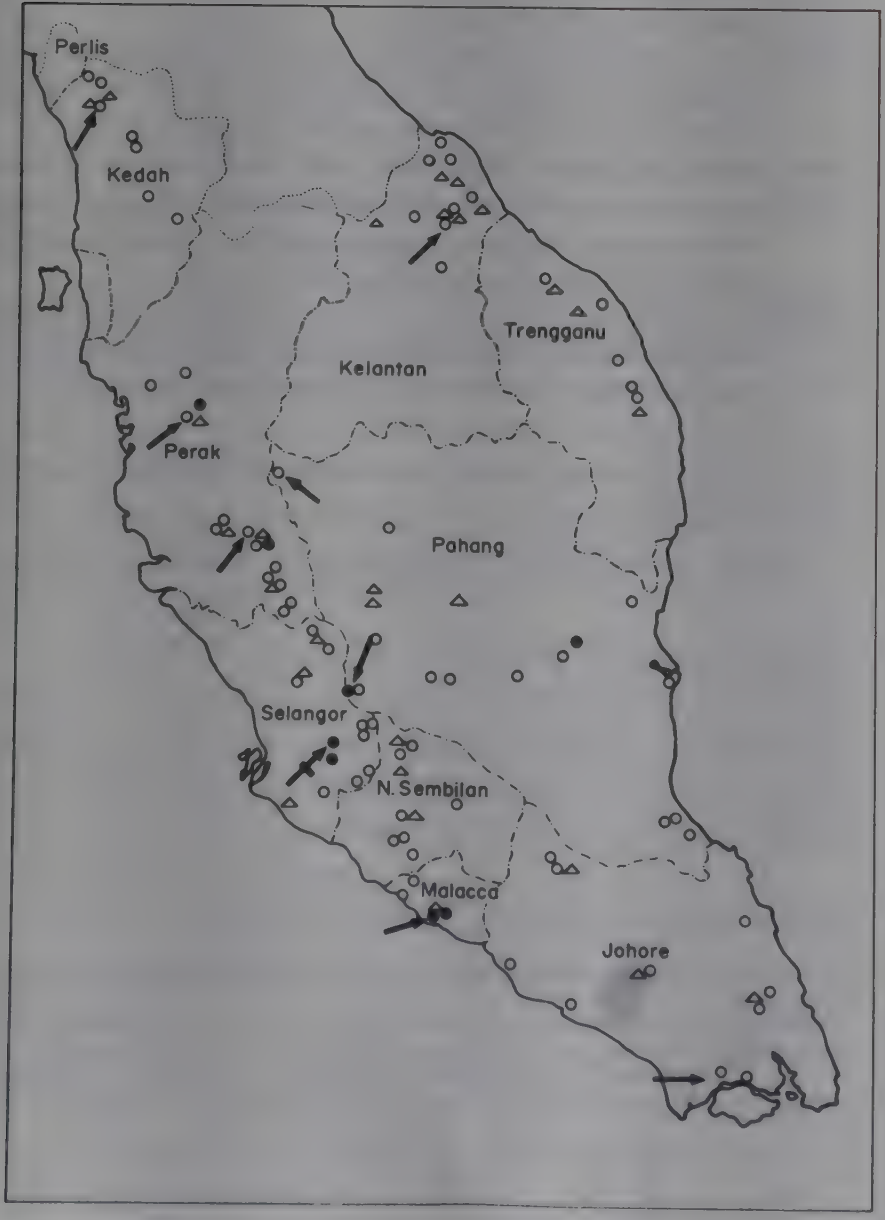

Fig. 1. Distribution of *Lernaea* spp. in Peninsular Malaysia. ○ Presence of *L. cyprinacea;* ● Presence of *L. cyprinacea* and *L. polymorpha;* △ Absence of lernaeoisis; ↗ landing stations and ↗ importing agency.

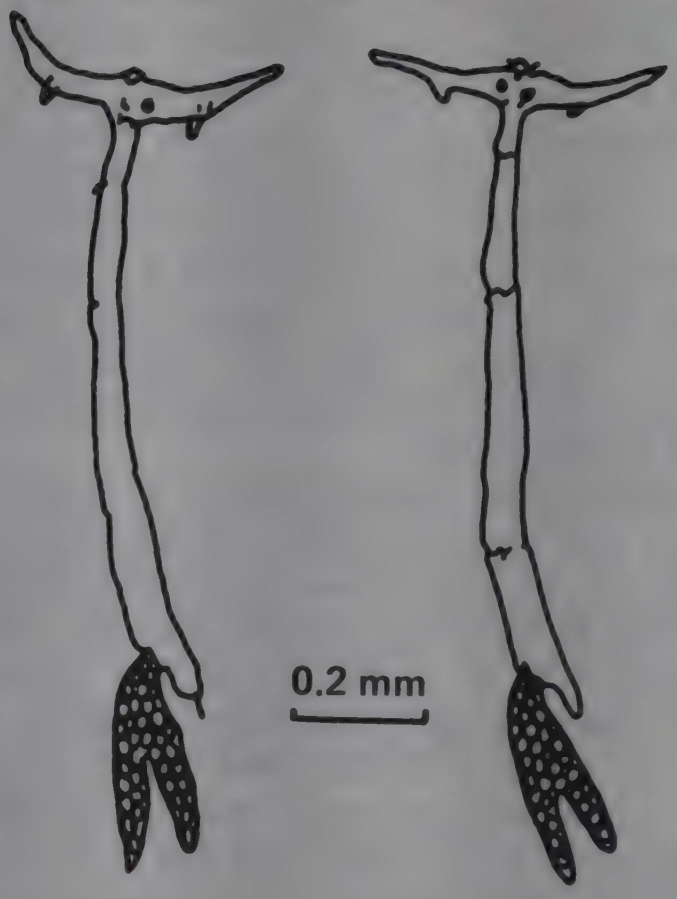

Fig. 2. *L. polymorpha* from *A. nobilis.*

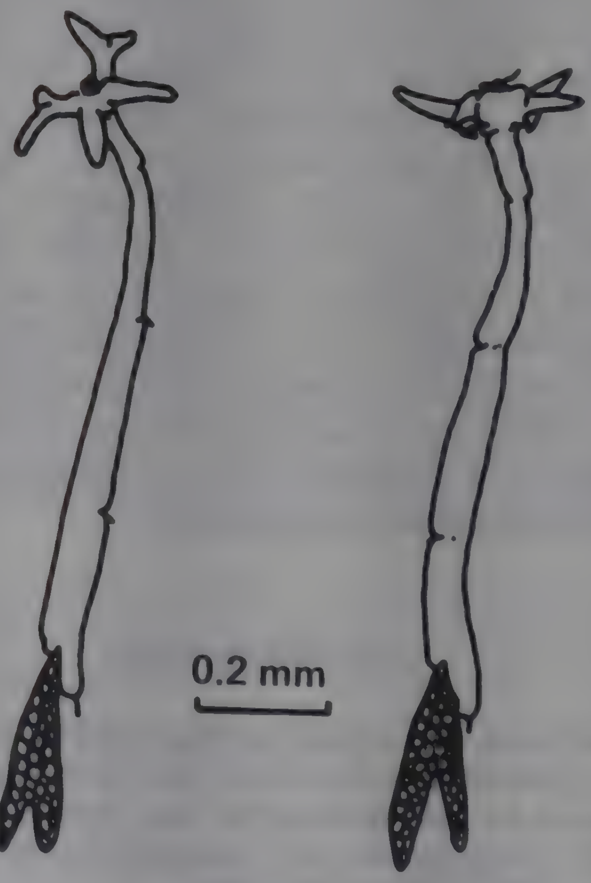

Fig. 3. *L. cyprinacea* "Asian" form from a wide range of hosts.

The Life Cycles of *Lernaea polymorpha* and *L. cyprinacea*

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Abstract

The life cycles of two fish parasites, *Lernaea polymorpha* and *L. cyprinacea* were determined under laboratory conditions. Experiments were conducted with four replicates. *Aristichthys nobilis* was used as host for *L. polymorpha* while *Carassius auratus*, *Helostoma temmincki* and *A. nobilis* were used as hosts for *L. cyprinacea*. The life cycles of *L. polymorpha* and *L. cyprinacea* were similar irrespective of the host species. The eggs from both species which hatched into nauplii required 14 to 15 days to form young female parasites. Egg development proceeded through three stages of nauplius and five stages of copepodid before reaching the adult male or young female stage. Water temperature ranged from 24.3 to 29.0°C. The two species of parasites could not be differentiated morphologically at the larval stages. In the absence of a host, the parasites developed to copepodid stage I only and died on the third day of that stage. These results are compared with the findings of other studies. A schedule for treatment of lernaeosis is also discussed.

Introduction

Of the two *Lernaea* species identified in Peninsular Malaysia, only the life cycle of *L. cyprinacea* has been studied extensively, e.g., by Wilson (1918) and Shields (1976) from the United States; Yashouv (1959), Lahav and Sarig (1964) from Israel; Grabda (1963) from Poland; Bauer et al. (1973) from Russia; Al-hamed and Hermiz (1973) from Egypt and Rukyani (1975) from Indonesia. The life cycle of the less commonly known parasite *L. polymorpha* has only been described in China by Pan et al. (1979).

This study was conducted to establish the life cycles of *L. cyprinacea* and *L. polymorpha* at local water temperatures and to compare various aspects of the life cycles. Adults of *Lernaea* show only minor differences, and identification difficulties are compounded by the polymorphism exhibited by *L. cyprinacea* (Shariff 1985). Differences were therefore sought in the larval stages. As with morphological adaptation, many parasites exhibit physiological adaptations, and these might be reflected in the duration of the different stages of the life cycle at certain temperatures. Such information might be useful in identifying larvae to species level in the absence of adult stages or whenever they cannot be separated morphologically. The potentials for polymorphism in the larval stages were examined by using three different fish hosts.

Materials and Methods

A constant supply of the parasites *L. polymorpha* and *L. cyprinacea* was maintained at the Faculty of Fisheries aquarium for the study. The original material was obtained from the university ponds where infected carp, *Aristichthys nobilis*, were collected. The parasites were identified to species level based on the key prepared by Harding (1950).

L. polymorpha was maintained on *A. nobilis* while *L. cyprinacea*, which has a wider range of host susceptibility, was maintained on goldfish, *Carassius auratus*. Thirty fish were kept in each tank to maintain a constant culture of both species of the parasite. Fish which recovered from the infection and which were suspected of becoming immune to the infection (Shariff 1981) were removed and replaced with naive fish.

The experiment was designed to study the life cycles of *L. polymorpha* on *A. nobilis* and of *L. cyprinacea* on *C. auratus*, *H. temmincki* and *A. nobilis*. Thirty fish of each species were used. The standard lengths of the fish were as follows; C. auratus, 10-18 cm; *Helostoma temmincki*, 11.5-18 cm; and two batches of A. nobilis, 10-15 cm. The fish were treated with formalin at 166 ppm for a half hour and quarantined for two weeks to ensure that they were free of Lernaea and other ectoparasitic infection. Each group of fish was then introduced into glass tanks of 107 x 46 x 46 cm fitted with undergravel filtration systems.

Eggs of *L. polymorpha* and *L. cyprinacea* were obtained from the stock culture. To ensure that the egg sacs used were of the same age group, the development of the eggs was synchronized by removing the second batch of egg sacs from all the parasites simultaneously as suggested by Shields (1976). Eggs were collected from the third batch of eggs of each parasite species and placed in petri dishes. Thirty egg sacs were collected to establish each host-parasite system. The eggs were observed constantly and the hatching time was recorded.

When the majority of the eggs were hatched, the nauplii of *L. polymorpha* and *L. cyprinacea* were introduced into tanks to establish their respective host-parasite system. Larvae of both parasite species were also kept in separate petri dishes to study the developmental stages of the parasite without a host. The petri dishes were left uncovered and kept in the aquarium shed along with the fish tanks.

The development of the parasites on the fish in the tanks was monitored by taking samples twice daily at 9 a.m. and 4 p.m. Collection of the larvae was made with a special bottle designed for the purpose (Fig. 1). The bottle was drawn gently across the fish tank to collect free swimming stages of the parasite. With the progressive development of the larval stages it became more difficult to obtain the copepodid by the above method. Parasites in the copepodid stage normally attach themselves temporarily to the host for feeding (Kabata 1970); they are best collected by dipping the fish in 10% formalin and collecting the released larvae. The larvae in the petri dishes were collected with a Pasteur pipette. The samples collected were kept in 10% formalin and later mounted on slides in formalin and examined under a microscope. The development of the preadult stage which was partially embedded in the host tissue was made by dissecting the parasite from the tissue. Four replicates of the experiment were conducted for each treatment. The maximum and minimum diurnal water temperatures were recorded daily.

Results

There were no differences between the durations of the various stages of the life cycles of *L. polymorpha* and *L. cyprinacea* obtained from their respective hosts used in this study (Table 1). The development of both species of the parasite from hatching to adult stage took an average of 14-15 days at a mean temperature of 27°C. The diurnal temperature recorded during the study period was 24.3-29°C.

There were three naupliar stages, nauplius I, II and III, and five copepodid stages, copepodid I-V. the copepodid female developed to the cyclopoid stage (premetamorphosis female) which embedded in host tissue and finally metamorphosed into the sedentary female parasite. The male cyclopoid died after 24 hours. The detailed morphological structure of the different stages is shown in Figs. 2 and 3. Each nauplius stage lasted for only 24 hours so that nauplius III was observed on the third day. The successive development of copepodid I to copepodid V took a further seven days. Each copepodid stage lasted an average of two days.

The cyclopoid was obtained on the 12th or 13th day. At this stage the female parasites were seen to be firmly attached to the host tissue. The females underwent metamorphosis to form the young sedentary parasite within 12 hours. The young parasites were thin and transparent and difficult to observe without the aid of a microscope or magnifying lens. A pair of small milky white egg sacs could be seen on the recently attached parasite. The milky white eggs gradually turned to a green color within 24 hours. At this stage the embryos were fully developed. After the formation of the embryo the eggs hatched within the next 12 hours. A second batch of eggs appeared within 12-24 hours after the first batch of eggs had hatched.

The eggs which hatched in the petri dishes without a host developed into the third nauplius stage and the first copepodid stage within the same time period as those larval stages on a host (Table 2). No parasites were seen to develop further than the copepodid I stage in the absence of a host, and they died on the seventh day after hatching, that is, on the third day of the first copepodid stage.

Discussion

The life cycles of both *L. cyprinacea* and *L. polymorpha* were successfully completed in the experimental systems used. The life cycles of the parasites were identical at the mean temperature of 27°C and therefore the two species could not be separated on this basis.

The need for an intermediate host species to complete the life cycle of *Lernaea* was initially reported by Wilson (1918). This was further indicated by Fryer (1961) when he found that the copepodid stages of *L. cyprinacea* infected *Bagrus docmac* which served as an intermediate host before they infected *Tilapia*. Similarly, Thurston (1969) reported that *L. barnimiana* lived on *Tilapia* and *Haplochromis* as an adult and required *Bagrus* as an intermediate host in the life cycle. These reports were from studies of fish in their natural habitat. However, in this study only one host species was used in each individual tank to complete the life cycles of both *L. cyprinacea* and *L. polymorpha*, which indicated that the use of the intermediate host was flexible and revealed the adaptability of the parasites.

The results of the study on the development of the nauplius to cyclopoid stage agrees closely with that of

Yashouv (1959) and Shields (1976). At 26-29.5ºC Yashouv reported that development of *L. cyprinacea* from nauplius to cyclopoid stages was accomplished in 12-16 days, and according to Shields this took between 11 and 15 days at 25-32ºC which is close to the present study's finding of 12-15 days at 24.3-29ºC. However, according to Pan et al. (1979) the development of the nauplius and copepodid required only seven days at 26-31ºC.

Completion of the life cycle from hatching of the egg to the emergence of the adult parasite with its eggs ready to hatch took 16-17 days at 24.3-29ºC. According to Lahav and Sarig (1964) and Shields (1976), the life cycle of *L. cyprinacea* took 18-21 days at 25ºC and 18-25 days at 25-32ºC, respectively, while Bauer et al. (1973) reported that it took 25 days at 20ºC and 16-1/2 days at 30ºC. Rukyani (1975) found that at 28ºC it took 21-23 days. The development rate was clearly sensitive to temperature but variation within the reported range 25-32ºC was as great as eight days. The large variation in the number of days it took to complete the life cycle over a small temperature range could be due to the differences in the mean and the durations of maximum and minimum temperatures at which the studies were conducted.

Thurston (1969) found that *L. barnimiana* required 15 days to develop to cyclopoid stages at 21-26ºC. This also closely resembles the findings for *L. cyprinacea* and *L. polymorpha* in the present study, indicating a similar pattern of development within the genus *Lernaea*.

In the absence of a host, copepodid I died on the third day without undergoing further development. This is in agreement with Shields and Tidd (1968) although their experiment was conducted at 28ºC as compared to a wider range of temperatures of 24.3-29ºC recorded during the present study. The inability of the copepod I to survive and molt indicates that it is an infective stage and is incapable of surviving without a host. This information suggests the possibility that a satisfactory elimination of *Lernaea* from an infected site is possible by leaving the pond fallow for a minimum period of seven days until all the infective stages have died.

It would not be necessary to identify the parasite to species level before preparing the treatment schedules since the similar life cycle would require a similar schedule for both *L. cyprinacea* and *L. polymorpha*. The treatment of the parasite with organophosphate compounds is not effective at the free swimming naupliar stages (Kabata 1970; Shariff et al. 1986) or the adult parasites which are embedded in the host tissue. The treatment schedule would therefore be directed to kill the free swimming copepodid stages. At mean temperatures of 27ºC the copepods take from eight to nine days before they develop into the cyclopoid stage and it would therefore be necessary to schedule the treatment before the eighth day. Thus, to ensure a successful treatment it would be advisable to introduce the drug at seven-day intervals (providing a safety margin of one day before the parasite embeds in the host tissue) until the infection has cleared.

Acknowledgements

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Table 1. Development of *L. cyprinacea* and *L. polymorpha* on their respective host.

No. of days	*L. cyprinacea* *A. nobilis*	 *C. auratus*	 *H. temmincki*	*L. polymorpha* *A. nobilis*	Developmental stages
1	I	I	I	I	
2	II	II	II	II	Nauplius
3	III	III	III	III	
4	I	I	I	I	
5	I	I	I	I	
6	II	II	I and II	I and II	
7	III and II	II	II	II and III	Copepodid
8	III	III	III	III	
9	III and IV	IV	IV	III	
10	IV and V	V	IV and V	IV	
11	V	V	V	V	
12	V and cyp	V and cyp	V and cyp	V and cyp	
13	cyp	cyp	cyp	cyp	Cyclopoid
14	cyp and young female	cyp and young female	cyp and young female	cyp and young female	(Premetamorphosis female and adult male)
15	young female	young female	young female	young female	(Postmetamorphosis female)

Table 2. Development of *L. cyprinacea* and *L. polymorpha in vitro.*

No. of days	*L. cyprinacea*	*L. polymorpha*	Development stages
1	I	I	
2	II	II	Nauplius
3	III	III	
4	I	I	
5	I	I	Copepodid
6	Died	Died	

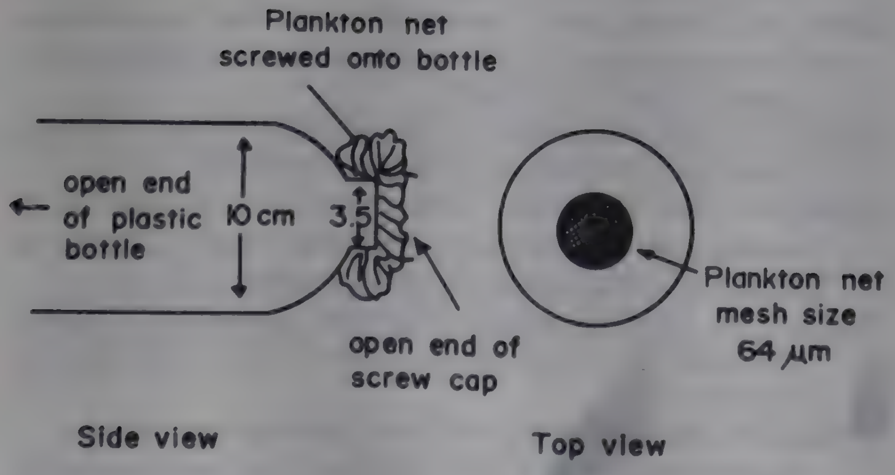

Plankton net
screwed onto bottle
open end
of plastic
bottle
10 cm
3.5
open end of
screw cap
Plankton net
mesh size
64 µm
Side view
Top view

Fig. 1. Modified plastic bottle for sampling larval stages of *Lernaea.*

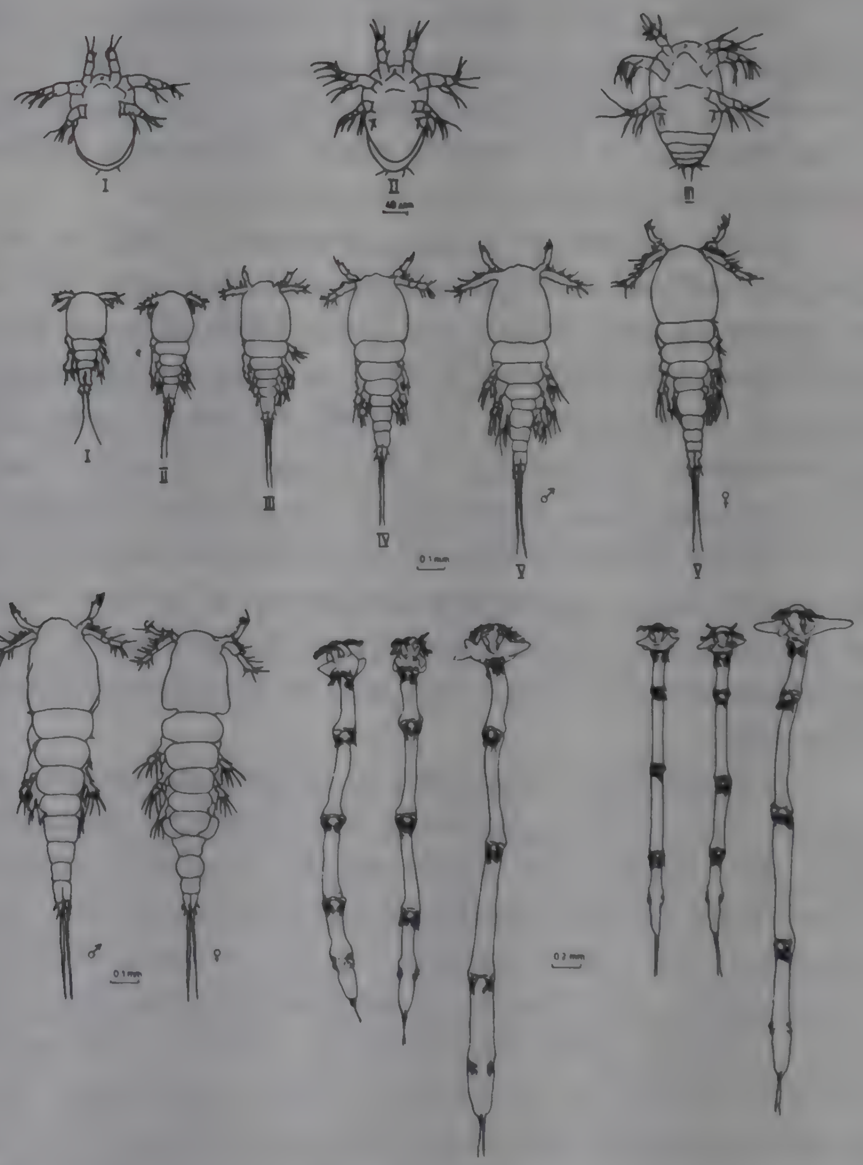
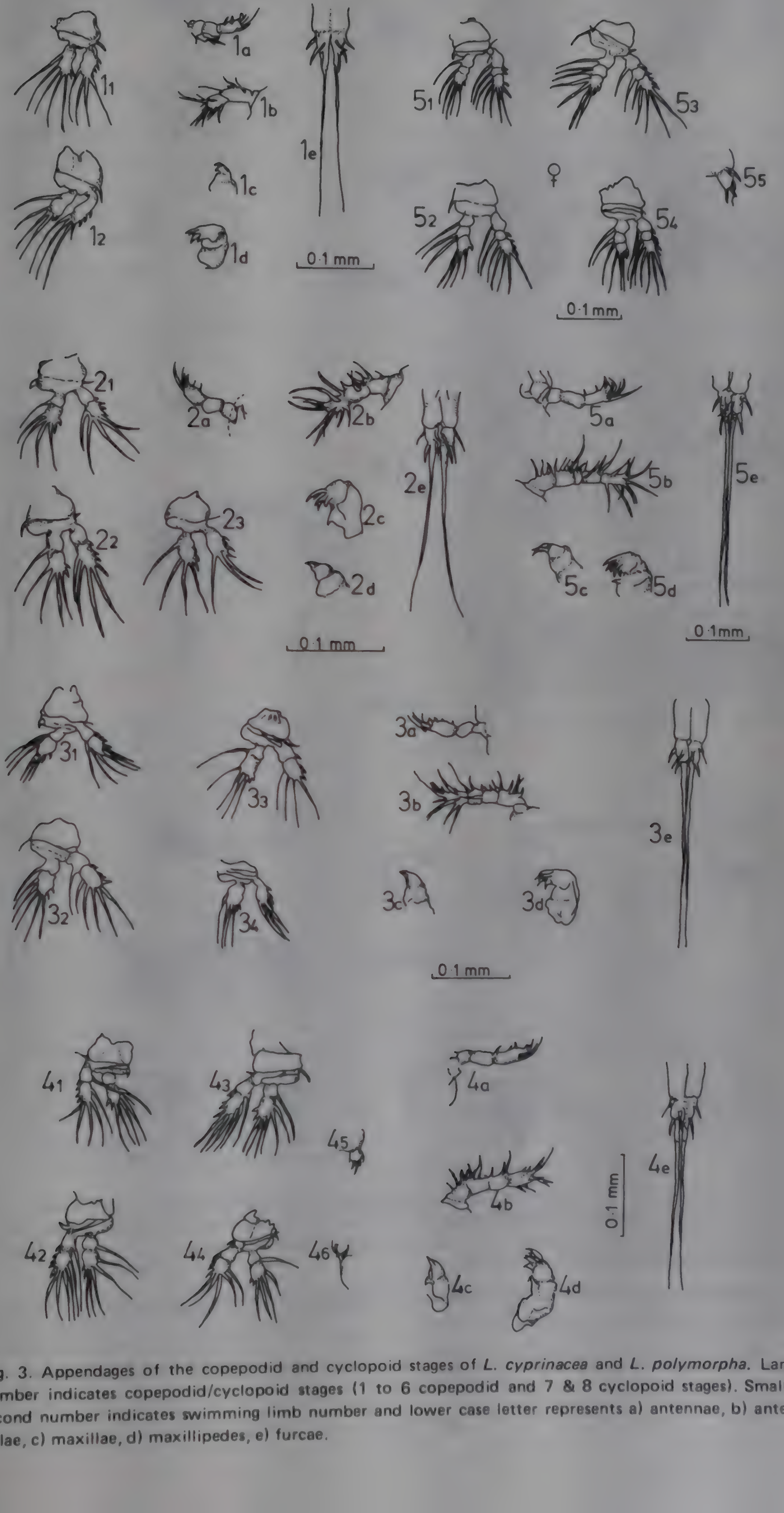

I
II
III
I
II
III
IV
♂
V
♀
V
♂
♀

Fig. 2. Developmental stages* of *L. cyprinacea* and *L. polymorpha.*

*Since the copepodid and cyclopoid stages of *L. cyrinacea* and *L. polymorpha* were similar the figures represent both species.


1_1
1a
1b
1c
1d
1e
1_2
0·1 mm
5_1
5_3
♀
5_5
5_2
5_4
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2_1
2a
2b
2e
2c
2d
2_2
2_3
0·1 mm
5a
5b
5e
5c
5d
0·1mm
3_1
3_3
3a
3b
3e
3_2
3_4
3c
3d
0·1 mm
4_1
4_3
4a
4_5
4b
0·1 mm
4e
4_2
4_4
4_6
4c
4d

Fig. 3. Appendages of the copepodid and cyclopoid stages of *L. cyprinacea* and *L. polymorpha*. Large number indicates copepodid/cyclopoid stages (1 to 6 copepodid and 7 & 8 cyclopoid stages). Smaller second number indicates swimming limb number and lower case letter represents a) antennae, b) antennulae, c) maxillae, d) maxillipedes, e) furcae.

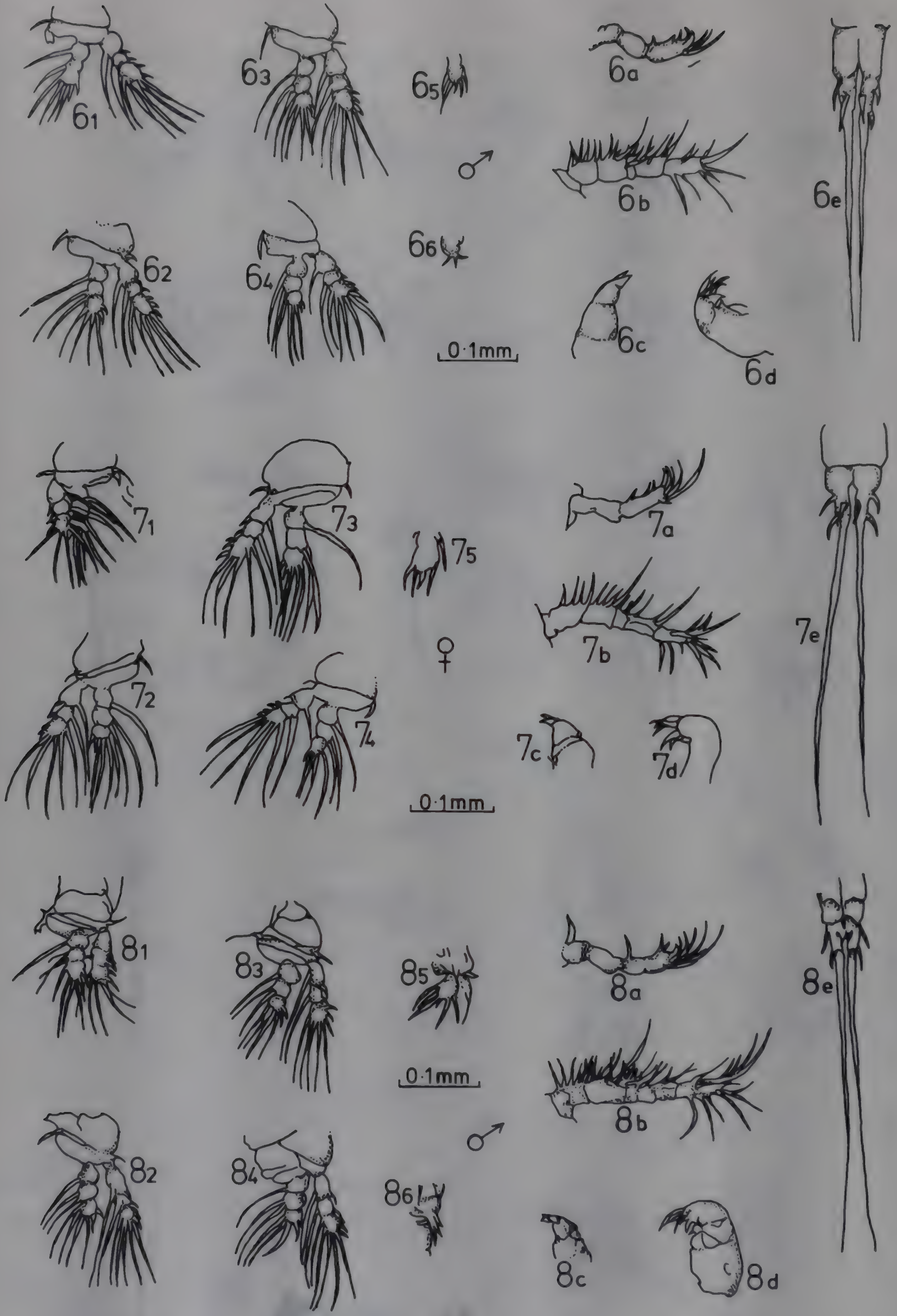
61
63
65
6a
♂
6b
6e
62
64
66
0·1mm
6c
6d
71
73
75
7a
♀
7b
7e
72
74
0·1mm
7c
7d
81
83
85
8a
8e
0·1mm
8b
♂
82
84
86
8c
8d

Fig. 4. Appendages of the copepodid and cyclopoid stages* of *L. cyprinacea* and *L. polymorpha*. Large number indicates copepodid/cyclopoid stages (1 to 6 copepodid and 7 & 8 cyclopoid stages). Smaller second number indicates swimming limb number and lower case letter represents a) antennae, b) antennulae, c) maxillae, d) maxillipedes, e) furcae.

*Since the copepodid and cyclopoid stages of *L. cyrinacea* and *L. polymorpha* were similar the figures represent both species.

Acquired Immunity of *Oreochromis mossambicus* to the Ciliate Ectoparasite *Ichthyophthirius multifiliis* (Fouquet)

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Abstract

The holotrichous ciliate *Ichthyophthirius multifiliis* is an important parasite of cultured fishes. The ability of *Oreochromis mossambicus*, a mouthbrooding cichlid, to mount an immune response to this parasite was investigated. Fish weighing 120-200 g were subjected to a trickle, sublethal infection of tomites (the infective stage of the parasite). Fish were free of *I. multifiliis* by day 18 after the initial exposure and remained free of the parasite up to nine months in an infectious environment. These fish were considered "effectively immunized." Another group of fish were injected with serum extracted from the "effectively immunized" fish. Fourteen days postinjection, fish were challenged by exposing them to a sublethal infection of tomites. The intensity of infection between injected and control fish was found significantly different ($P < 0.05$). Immobilization experiments showed that serum and skin mucus from "effectively immunized" fish immobilized tomites at much higher dilutions than that from nonimmunized fish. Ochterlony double diffusion tests carried out using the serum and skin mucus from immunized and nonimmunized fish against sonicated *I. multifiliis* whole cell antigen indicated the possible presence of soluble antibodies in the serum and mucus of immunized fish. An acquired specific immune response of humoral origin in this mouthbrooding tilapia is suggested and its implications for aquaculture discussed.

Introduction

Ichthyophthirius multifiliis is a ciliate protozoan ectoparasitic on freshwater fish. It is considered one of the most serious fish parasites (Bauer 1962; Meyer 1969; Pearson 1970; Hines and Spira 1973), which causes severe epizootics in wild (Elser 1955; Allison and Kelly 1963; Chappell and Owen 1969) and cultured (Butcher 1947; Johnson 1961; Bogdanova 1976; Valtonen and Keranen 1981) fish. During its infective stage, the tomite enters the fish's epithelium and produces a condition commonly known as "white spot" disease.

Fish have been shown to develop immunity to lethal levels of infection following exposure to sublethal levels of the parasite (Beckert and Allison 1964; Parker 1965; Hines and Spira 1974; Beckert 1975; Subasinghe 1982). This protection is believed to be due to the production of humoral antibodies (Hines and Spira 1974) and to the parasite antigen in the cilia (Goven et al. 1980).

Tilapias are commonly infected with *I. multifiliis* in culture systems. Although tilapias in general, including *Oreochromis mossambicus*, appear more resistant to ectoparasitic diseases than some other warm- and coldwater fish species, very little is known about their ability to mount an immune response to pathogens. In view of the significance of tilapias as cultured species, it was considered important to investigate their ability to mount an immunity response to pathogens such as *I. multifiliis*.

Materials and Methods

Genetically pure (as identified by McAndrew and Mujumdar 1983), hatchery-bred *O. mossambicus* weighing 120-200 g, not previously infected with *I. multifiliis*, were used throughout the investigation.

The parasites were propagated from a single isolate obtained from an infected cichlid, *Chichlasoma meeki*, and maintained by serial passage on *O. mossambicus* by introduction of uninfected fish into tanks where infected fish were held.

The standardized procedures described by Dickerson et al. (1981) were slightly modified and employed for the preparation and quantification of tomite suspensions used in experimental infections. The tomites were 6-12 hours old at the time of infection.

The 20 broodfish used were divided into four equal groups. Group A was used for the experimental infections, Group B for the experimental infections and collection of mucus and blood, group C as control and group D for the injection of immune serum and subsequent exposure to *I. multifiliis*.

Four days before Groups A and B were experimentally infected, skin mucus was collected from the fish in Groups B and C and concentrated by freeze-drying. The fish were also bled by cardiac puncture and 0.3-0.5 ml of blood was removed from each fish and the serum separated. Both the bleeding and the mucus collection were carried out under anaesthesia (benzocaine dissolved in acetone at 100 mg/l).

The four groups of fish were then placed in separate glass aquaria with 80 l of water. Each aquarium was individually aerated and a constant temperature of 27 ± 1°C was maintained. Groups A and B were then exposed to a sublethal dose level of 20,000 tomites/fish by adding an appropriate volume of tomite suspension of a known density. Groups C and D were maintained uninfected. All groups were fed on commercial trout pellets at 1% body weight/day. On the 12th, 24th and 30th days after initial infection, a tomite suspension estimated at a dose level of 20,000 tomites/fish, was added to Groups A and B facilitating a standard infectious environment.

Groups A and B fish were closely observed daily for the presence of visible *I. multifiliis*. Five-day random skin scrapings were carried out on individual fish to confirm the absence of parasites.

Both uninfected control fish (Group C) and Group B fish were individually bled at five-day intervals for 35 days and mucus was collected and concentrated on the 35th day.

Two fish from Group B were further maintained for nine months in an infectious environment and were bled and their serum separated after the ninth month.

Fish in the uninfected Group D were individually injected intraperitoneally with 0.5 ml of pooled serum from the immunized fish in Group B at day 35 postinfection. Fifteen days later, both Groups C and D were subjected to an infection of 20,000 tomites/fish. At five days postinfection the fish were individually anaesthetized and the visible white spots (trophonts) on the caudal fin counted under a sterio microscope.

Immobilization titres of 4 to 5-day old freshly-dislodged trophonts were measured using the individual serum samples and pooled mucus samples collected from Groups B and C. The methods described by Sonneborn (1950) were slightly modified for these tests.

Ochterlony double immunodiffusion tests were carried out using pooled serum and mucus collected from the fish of Groups B and C against killed sonicated *I. multifiliis* whole-cell antigen. Noble agar (Difco) (1%) was used and tests were replicated four times.

Results

All fish in Groups A and B were visibly infected with *I. multifiliis* at four to five days postinfection. The number of visible parasites began to decrease rapidly from day 12 and by days 14-15 no parasites were visible. Skin smears revealed that the parasites had completely disappeared by days 17-19 postinfection. All fish remained free of *I. multifiliis* under infectious conditions until the experiment ended and were considered "effectively immunized".

Fig. 1 represents the relationship between the log e mean serum immobilization titres and time for the five group B fish exposed to a sub-lethal infection of *I. multifiliis*. The titres rose from day 0 onwards, with a maximum rise between days 5 and 10 postinfection. The titres from the two fish from Group B held for 270 days postinfection and kept under infectious conditions gave a log e titre of 6.09 ± 0.30 (Fig. 1).

Table 1 describes the parasite immobilization strength of the mucus and serum from the fish used in Groups B and C. The nonimmune control fish (Group C) serum resulted in less than 50% immobilization at all dilutions below 1:2. In contrast the immune fish (Group B) serum showed 50% or more immobilization at all the dilutions below 1:1,024. The pooled concentrated mucus fom the nonimmune control fish showed no degree of immobilization while pooled concentrated mucus from the immunized fish (Group B) gave 50% immobilization at 1:2 dilution.

No precipitation arcs were observed for serum and mucus of nonimmunized control fish against *I. multifiliis* antigen in Ochterlony immunodiffusion tests. In contrast, both pooled immune serum and mucus from fish in Group B at day 35 postinfection gave precipitation arcs against the antigen. There were no indications of more than one precipitation arc between any serum or mucus sample and *I. multifiliis* antigen.

The mean number of *I. multifiliis* counted on the caudal fin of group C fish was 113 ± 49. The Group D fish, injected with immune serum, showed a significantly lower ($P > 0.05$) number of parasites on the caudal fin, with a mean of 23 ± 11.

Discussion

Postinvasion immunity to *I. multifiliis* was first experimentally demonstrated in *Cyprinus carpio* L. by Hines and Spira (1974). Since then *Ictalurus punctatus* (Rafinesque) and *Salmo gairdneri* (Richardson) were also shown to develop immunity to the parasite by Beckert (1975) and Wahli and Meier (1985), respectively. This investigation demonstrates the presence of postinvasion immunity in *O. mossambicus*. It has clearly shown that *O. mossambicus*, recovering from a sublethal level of infection of *I. multifiliis*, is refractory to a normally lethal level of infection and that the immunity was retained for at least 270 days (the duration of the experiment) in an infectious environment.

Hines and Spira (1974) noted that *C. carpio*, exposed to a sublethal level of infection, developed a heavy infection which peaked at 16 days after the initial exposure. The parasite had completely disappeared from the skin and gills by 21 days postexposure. According to Wahli and Meier (1985), the fish were heavily infected three weeks after the initial infection. However, they did not report the time the parasites completely disappeared. In this study the parasites completely disappeared between 17 and 19 days.

Another obvious difference between this study and the two previously mentioned reports is the temperature at which the fish were maintained. Wahli and Meier (1985) used 11°C, Hines and Spira (1974) 20-23°C. The immune response in all ectothermic vertebrates is temperature-dependent and low temperature delays or completely abolishes the antibody production (Ellis 1978). The life cycle of the parasite is also temperature-dependent (Subasinghe and Sommerville, unpublished data). Thus the level of infection and the pathogenicity of the parasites on the fish could also vary at different temperatures and affect the response rate. These factors could account for the differences in the length of time the parasites completely disappeared from the immunized fish as reported.

Hines and Spira (1974) found a rise in serum immobilization titres in immunized carp between days 10 and 22, the period when all the parasites diappeared. In this study, the most rapid increase in the serum immobilization titres was between days 5 and 10. The comparatively early immune response indicated by this early rise in serum immobilization titres may be associated with possible differences in the infection levels employed. The dose level used by Hines and Spira (1974) is not directly comparable with that used in this study due to the differences in measuring dose levels, but is considered a high dose compared to that of this study.

According to Ingram and Alexander (1980), for teleosts in general, the average time for the appearance of the antibody-secreting cells (ASC) after antigen injection is about eight days, with maximum time about 16-18 days. However, Sailendri and Muthukaruppan (1975) found that the peak production of ASC in *O. mossambicus* was in five days postimmunization. Although this difference could be attributed to the rearing temperature, it may also be due to specific differences between fish.

The presence of precipitating antibodies has been demonstrated in various species of fish naturally infected with intestinal parasites (Harris 1972; Cottrell 1977; McArthur 1978). However, these antibodies did not afford any protection. In contrast, this study clearly indicates the presence of specific precipitating antibodies in the serum and mucus which effectively protected *O. mossambicus* immunized against *I. multifiliis*. The original suggestion of Fletcher and Grant (1969) that an active secretory system moves immunoglobulins out of the blood into the mucus is supported by this investigation. The mechanism of host protection appears to be based on the immobilization of the parasite by soluble antibodies at the site of host-parasite contact or perhaps even after the parasite has entered the epithelium.

Although the immunization of fish by injection of killed *I. multifiliis* or related ciliate antigens has been demonstrated by Goven et al. (1980, 1981), no reports were found on the passive immunity by injection of immune fish serum. This study demonstrated that passive immunity by injection of immunized serum is possible. Similar protection was demonstrated by Harrell et al. (1976) in rainbow trout injected with trout anti-*Vibrio angullarum* serum against vibriosis. Although the present study did not cover an examination of the duration of the passive immunity in *O. mossambicus*, the fish was evidently still strong at the 20th day postinjection when the challenge was presented. However, protection was not complete, as all five serum-injected fish sustained mild infection. In contrast, fish exposed to sublethal infection of *I. multifiliis* did not have parasites after 20 days. Therefore, the acquisition of immunity through exposure to sublethal infections was more effective than the passive immunity conferred by injection of immune serum.

The ability of the fish to retain immunity over the observed nine-month period in an infectious environment could be of some importance to aquaculturists. This suggests that when fish are kept in relatively poor husbandry conditions where recurrent host-parasite encounters are likely, they are able to remain refractory to *I. multifiliis* provided that the initial exposure was sublethal. Although there is no effective vaccine developed yet, fish, especially valuable broodstock, could be protected by careful manipulation of well controlled infection regimes in environments where the parasite is enzootic.

This deliberate exposure of healthy fish and fingerlings to infection by *I. multifiliis*, however, would be a rather extreme measure. It would be effective in stimulating immunity, but the stress would inevitably adversely affect growth. The production of a vaccine which could be administered by bath, injection or orally is expensive and not cost-effective for most low-technology culture operations. At this stage, parasite control during natural outbreaks which can prevent lethal infections and enhance the fish's natural immunity would seem the only realistic method. Research on the ability of immune females to protect their progeny, currently being undertaken by the authors, is a further development towards solving problems of fish parasitology.

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Table 1. Parasite immobilization strength of the serum and mucus of five effectively immunized *O. mossambicus*.

Sample	Dilution	Immobilization Fish 1	Fish 2	Fish 3	Fish 4	Fish 5
Non-immune serum	1:1	****	***	***	***	***
	1:2	**	**	**	**	**
	1:4	*	*	*	—	—
	1:8	—	—	—	—	—
Immune serum	1:128	****	****	****	****	****
	1:256	****	****	****	****	****
	1:512	****	***	****	****	****
	1:1,024	***	**	***	***	***
	1:2,048	*	—	*	*	*
Non-immune pooled mucus	1:1	—				
	1:2	—				
Immune pooled mucus	1:1	**				
	1:2	**				
	1:4	*				
	1:8	—				
	1:16	—				

**** = 100% immobilization in 3 hr
*** = 75% immobilization in 3 hr
** = 50% immobilization in 3 hr
* = 25% immobilization in 3 hr
— = no immobilization in 3 hr

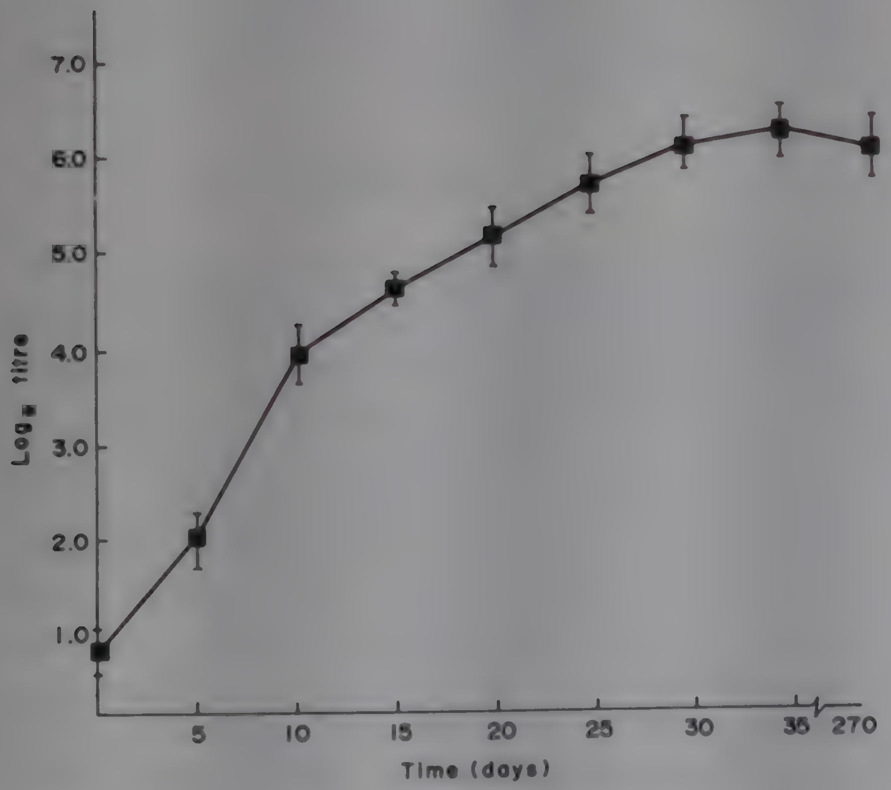

Fig. 1. Pattern of change of mean serum immobilization titre ± (S.D.) of five *O. mossambicus* exposed to trickle sublethal infection of *I. multifiliis*.

Effects of Oil on Mangrove Organisms

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Abstract

The acute effects of oil on shrimp *(Penaeus monodon)*, snail *(Cerithidea* sp.*)* and cockle *(Anadara* sp.*)* in mangrove forests under a wide range of ecological conditions, i.e., static, flow-through and *in-situ* habitats were monitored. These organisms were observed to be more susceptible to chemically-dispersed than naturally-dispersed oil in static condition and less in *in-situ* condition. This is in stark contrast to the effects of dispersed and undispersed oil on mangrove flora; i.e., the chemically-dispersed oil was less toxic at all levels of the experiment. The overall growth of the surviving shrimp in the oil-impacted habitats seems compatible with that in the unpolluted, aquacultural pond although the initial growth was slightly retarded. A hypothesis was offered to explain this discrepancy. The various experiment designs are detailed to provide a link between a highly-artificial laboratory condition and a realistic field situation. The article also attempts to answer: "To what extent has the oil polluted the mangrove environment in Penang?"

Introduction

There is good deal of debate on the types of system to monitor or test the toxicities of pollutants, including oil and dispersants. Biologists tend to look for a highly static system which will produce a very stringent set of standards. Usually, this highly static system may be reproduced easily in laboratories. Technologists and development-oriented planners would argue for a more open flow-through system closer to a "real world" situation. As a rule, this open system tends to yield a set of data quite variable, not to mention its reproducibility. The tone of such debate persisted as Lindblom (1978) criticized some of the static laboratory testing techniques. He opined that static laboratory tests were too artificial and the results should not be used as a basis to interpret field occurrences. The major fault of laboratory tests is the lack of water exchange. Southward (1982), on the other hand, rapped those who belittled the effect of oil and oil dispersants on the environment. To him the safety margin in dilution factors cannot mitigate the subtle effects in the field. Results of laboratory investigations are borne out by evidence from the field. Due to the various criticisms directed at laboratory tests, some improvement of the test design, notably by Mackay et al. (1978), has been made.

This study tried to devise a series of experiments ranging from a highly reproducible static to an exceedingly variable field trial. This design may seem time-consuming, but it enables planners and regulatory agencies to make appropriate decisions to lessen the damage of a particular chemical in an environment under a particular condition.

Materials and Methods

Experiments were carried out at five levels, namely: (a) static condition; (b) partially flow-through system; (c) fully flow-through system; (d) *in-situ* field condition; and (e) 'real world' situation.

Experiments under the static condition were carried out in 10-l plastic containers with oil and dispersed oil throughout the entire experiment. A total of 40-60 *Penaeus monodon* postlarvae were conditioned for one day in the containers before the experiment. In the cockle experiment 10 carefully-selected *Anadara* sp. were planted on the mud surface before the test.

The partially flow-through system consisted of a series of 1-t circular fiberglass tanks. A mudflat was built in each tank and seawater was flushed out manually whenever necessary. Only *Phascolosoma* were counted following the toxicity test of dispersed and undispersed oil.

The fully flow-through system comprised four 6-t rectangular fiberglass tanks where a semidiurnal tide was simulated with timer-controlled pumps. A mudflat was also built in each tank. The LC_{50} at 48 hours of dispersed oil on *Penaeus* postlarvae was determined.

A series of zinc enclosures were used in the *in-situ* experiments. Each enclosure had a volume of about 100 l at the high water spring in the mangrove swamp. A total of 16 *Cerithidea* snails were placed in each enclosure.

In the 'real world' oil spill experiment 90 l of oil was spilled over an area of 9 m^2 in the mangrove swamp 1 hour before high tide. This was repeated using 90 l of dispersed oil in another similar area of 9 m^2.

The crude oil used was Arabian light and the dispersant Corexit 9527. All the experiments were carried out at ambient temperatures.

At levels 1-3, oil and dispersed oil were applied to the systems, imitating high water. At levels 4 and 5, oil and dispersed oil were spilled when the mangrove swamp was exposed at low tide.

The toxicities of oil and dispersed oil may be estimated in many ways. One of the most frequently used methods is to estimate the lethal concentration of oil, killing 50% of the organisms at 96 hours, i.e., LC_{50} (96 hours). The toxicity (LC_{50} at various exposure times) of dispersed and undispersed oil was estimated by the probit analysis similar to that of Roberts and Boyce (1972) to establish dose/mortality relationship between dispersed oil and cockle, *Anadara* sp. (Fig. 1). The oil concentration in this article is defined as the volume of oil per volume of water (v/v), but not the amount of oil dissolved in water. For example, at 10,000 ppm (v/v) the actual dissolved hydrocarbon is estimated at about 50 ppm.

Results and Discussion

The merit of using multitiered experiments in determining the toxicities of oil and dispersed oil was discussed at length by Lai (1984). To issues are of primary concern. First, the impact of oil and dispersed oil on mangrove itself was slight. For example, the toxicity (LC_{50} at 30 days) of Arabian light to *Avicennia* in the field was estimated at 1,400 ppm while the toxicity (LC_{50} at 30 days) of chemically-dispersed oil was at 6,417 ppm. These toxicities were exceeded by those of oil and dispersed oil to the invertebrates, particularly shrimp, by a 2:3 order of magnitude. That is to say by the time the oil or dispersed oil killed 50% of the mangrove, many invertebrates had long been killed. The alternative argument is that as long as the mangrove remains intact, it will recover through depuration and recolonization of the swamp by invertebrates will follow.

Second, the present design of a multitiered approach enables one to observe behavior patterns of the mangrove fauna such as vertical avoidance and emergence from oil-impacted habitats. The design also clearly shows the changing environment from a purely static laboratory condition to field situation.

Table 1 illustrates the impact of oil and dispersed oil on various groups of invertebrates in the mangrove swamp. At level 1 the LC_{50} of undispersed Arabian light on *Anadara* at 96 hours exceeded 50,000 ppm, while the toxicity (LC_{50} at 96 hours) of the chemically-dispersed oil was about 12,000 ppm. The undispersed oil was at least four times less toxic than the chemically-dispersed oil in static condition. The ratio of toxicity between undispersed and dispersed oil to the same organism at 114 hours increased by almost 20 times in favor of undispersed oil; the LC_{50} of the latter remained at a level above 50,000 ppm. At level 1, the toxicity of undispersed oil to *Penaeus monodon* was also about six times less than that of the chemically-dispersed oil. Thus, the toxicities of undispersed and dispersed oil to other organisms at the other levels of investigation can be compared. It should be mentioned that the toxicity (LC_{50}) at 48 hours of oil to *P. monodon* approximates 13-25 ppm reported by Brodersen et al. (1977), Anderson et al. (1981) and Rice et al. (1977).

In general, toxicity differences between undispersed and dispersed oil gradually narrowed to about 1.5 times from level 1 to level 5. In other words, the toxicity ratio of undispersed and dispersed oil approaches unity in the field condition. The gradual reduction in the differential impacts of naturally and chemically dispersed oil on mangrove fauna may also explain the astonishing growth recovery of *Penaeus monodon* at level 3 (Fig. 2). This seems to partially support the observation that as long as the mangrove vegetation remains intact, the invertebrates have a good chance to recover from the oil-impacted habitat. But if the toxicity results at level 1, especially those derived from tests on mangrove fauna, are considered, the impact will be frightening.

This finding contrasts with observations on the toxicities (LC_{50} at 30 days) of undispersed and dispersed Arabian light on mangrove saplings (Table 2). Lai and Feng (1985) observed that the toxicity of undispersed oil to mangrove was consistently higher than that of the chemically-dispersed oil at all levels of investigation. The toxicity ratio of oil and dispersed oil varied from 0.1 to 0.6; the mangrove flora became more tolerant to oil, both dispersed and undispersed, in the field rather than in the laboratory.

The difference in the relative impact of oil and dispersed oil on mangrove flora and fauna were analyzed with the Kolmogorov-Smirnov goodness of fit to decide on the hypotheses:

H_0: There is no difference in the relative impact of oil (a) and dispersed oil (b) on mangrove flora and fauna.
H_A: There is a difference in such an impact.

The data (a/b) are rearranged to facilitate the Kolmogorov-Smirnov analysis based on:

$$D_i = |\,\mathrm{rel}\,F_i - \mathrm{rel}\,\hat{F}_i\,| \text{ and } D_i' = |\,\mathrm{rel}\,F_{i-1} - \mathrm{rel}\,\hat{F}_i\,|$$

The test statistic $D = \max\ [(\max\ D_i),(\max\ D'_i)]$, whichever is larger, will then be compared with the critical values $D\alpha,n$ in the Standard Table to decide whether the H_0 hypothesis should be rejected or not at the α level of significance. H_0 was rejected when all the a/b ratios (0.1, 0.2, 0.5, ... 4, 6, 19) in Tables 1 and 2 were tested together, as the observed D = 0.642 was greater than the critical value $D_{0.05, 11} = 0.39122$ at $\alpha = 0.05$; this implies that there is a difference in the relative impact of oil and dispersed oil on mangrove organisms. However, if the a/b ratios (4, 6, 19) in the case of static treatment, i.e., level 1, of oil (a) and dispersed oil (b) to organisms are excluded from the analysis, H_0 cannot be rejected as the observed D = 0.325 is smaller than the critical value $D_{0.05, 8} = 0.45427$ at $\alpha = 0.05$ level of significance; this implies that the relative impact of oil and dispersed oil on mangrove organisms is not significantly different at levels 2, 3, 4 and 5 of the present investigation.

From the two analyses it appears that the static condition represents the main source of disparity. Mangrove fauna are particularly susceptible to chemically-dispersed oil under static condition (Table 1).

The above data provide useful indication of the underlying impact mechanisms of dispersed and undispersed oil against fauna and flora. Perhaps some emphasis can be given to oil pollution on faunistic distribution in order to provide a balanced viewpoint. Oil affects organisms by either passive deposition and oiling (Chan 1977) or active absorption of the light fraction (Anderson et al. 1974). It appears that both modes of action operate on mangroves, especially on young ones (Lai and Feng 1985). For taller, apparently older, plants the second mode played a predominant role as the sensitive organs, leaves and roots, lie physically outside the smothering range of the oil and dispersed oil. The roots, are well below the 6-10 cm depth, the maximum absorption range of oil by the soil in our study. The chemically-dispersed oil has ample time to be drained out of the habitat and as such is less toxic than the easily-trapped undispersed oil which slowly releases its toxic light fraction, leaving a lasting impact on the habitat. There is no evidence to support the argument that the pneumatophores are sensitive to the impact of oil as these organs were hardly formed at the time the oil spill occurred. When pneumatophores appeared in older plants there was hardly any mortality.

Many invertebrates have no protective mechanisms like the mangroves because they inhabit the first 10 cm of the soil sediment. Passive deposition and active absorption act upon the limited surface of the organisms and reinforce one another. Very often the undispersed oil floats on the surface of the water and takes longer to release its toxic light fraction to impact the organisms. On the other hand, the chemically-dispersed oil sinks faster and smothers them quicker too. It was often observed that the chemically-dispersed oil killed these organisms faster than the undispersed oil. It is possible that the composition of the respective cellular membranes also acts differently as physical barrier for the penetration of hydrocarbons. Steps are being taken to verify this hypothesis.

It seems reasonable to presume that at present the effect of oil on mangrove environment in Penang is slight. This conclusion seems to tally well with the observation that the level of dissolved hydrocarbons in coastal water off Muka Head at about 8-10 ppb (Feng, pers. comm.), is about 0.0003 times the impact toxicities reported in this article with an assumed LC_{50} at about 30 ppm.

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Table 1. Toxicities of oil and dispersed oil on the various invertebrates in mangrove swamps.

Level	Oil(a)	Dispersed oil (b)	a/b	Toxicity mode	Time (hr)	Organisms tested
1	> 50,000 ppm	12,342 ppm	> 4	LC_{50}	96	*Anadara*
	> 50,000 ppm	2,600 ppm	> 19	LC_{50}	114	*Anadara*
	36.7 ppm	< 6 ppm	> 6	LC_{50}	48	*P. monodon*
2	12,961 ppm	7,778 ppm	1.7	LC_{50} approx.	96	*Phascolosoma*
3	–	10.9 ppm	–	LC_{50}	48	*Penaeus monodon*
4	6,320 ppm	4,190 ppm	1.5	LC_{50}	48	*Cerithidea*
5	37,120 ppm	25,780 ppm	1.4	LC_{50} approx.	6-7 months	A multitude of species except *P. monodon*

Table 2. Toxicities of oil and dispersed oil on mangrove saplings.

Level	Oil(a)	Dispersed oil (b)	a/b	Toxicity modes	Time (days)	Species
1	–	–	–	–	–	–
2	77 ppm	770 ppm	0.1	LC_{50}	6	*Avicennia*
	1,296 ppm	2,333 ppm	0.6	LC_{50}	30	*Rhizophora*
3	–	286 ppm	–	LC_{50}	30	*Avicennia*
	–	7,884 ppm	–	LC_{50}	30	*Rhizophora*
4	1,400 ppm	6,417 ppm	0.2	LC_{50}	30	*Avicennia*
	2,790 ppm	5,860 ppm	0.5	LC_{50}	30	*Rhizophora*
	17,020 ppm	31,810 ppm	0.5	LC_{50}	30	*Bruguiera*

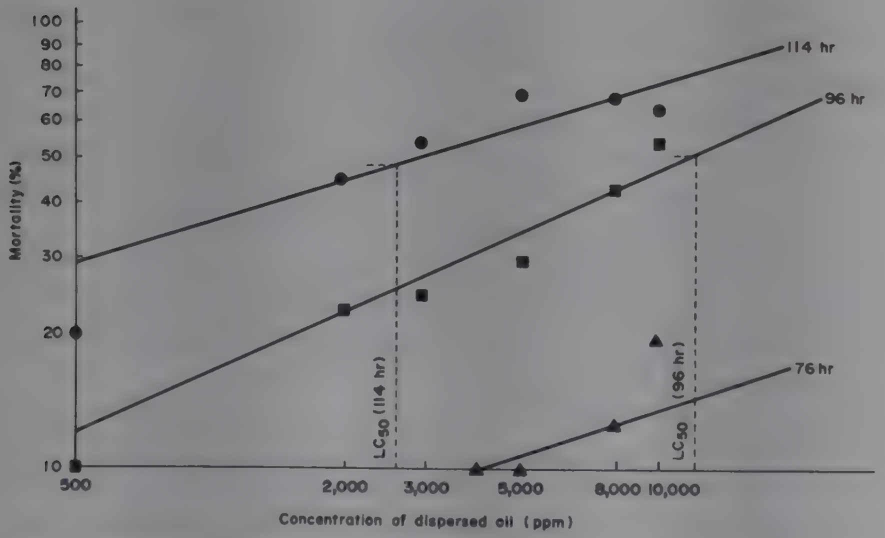

Fig. 1. Graphic probit analysis of cockle mortality and concentration of chemically-dispersed oil. 114 hr (●—●), 96 hr (■—■), 76 hr (▲—▲).

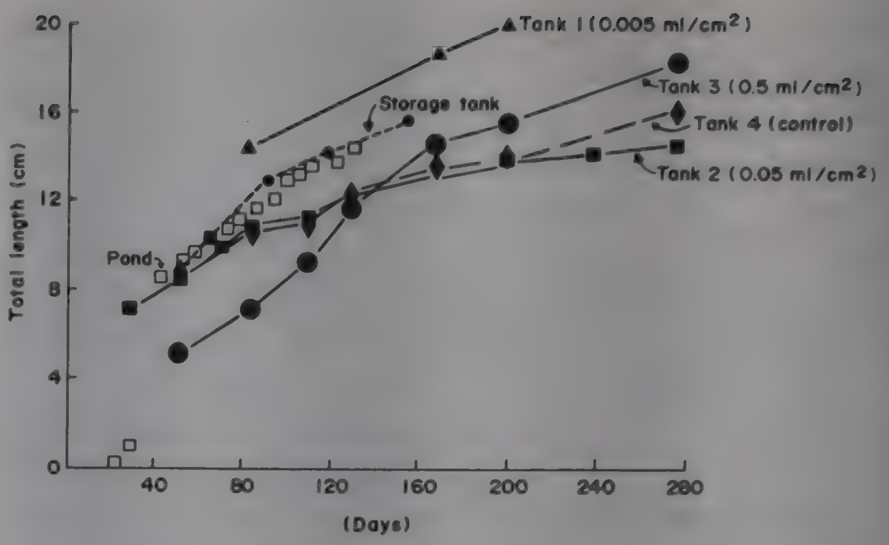

Fig. 2. *Penaeus monodon* impacted by various concentrations of chemically-dispersed oil.

Species Composition and Early Development of the Benthic Marine Algae in Relation to Water Pollution in Si Racha Bay, Chonburi, Thailand

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Abstract

The change in species composition and early development of benthic marine algae was investigated in relation to water pollution by the effluents from tapioca mills discharging into the coastal waters in Si Racha Bay, Chonburi, Thailand. The common species found growing along the coastline are *Enteromorpha flexuosa*, *Padina australis*, *Gracilaria crassa* and *Hypnea cornuta*, of which *P. australis* is most abundant.

The tetraspores of *P. australis* showed normal germination manner in seawater collected near the polluted area. The tetraspores germinate under broad ranges of salinity. Their germination rate is better in high salinities than in low salinities with the maximum of 31.8% at 32.2 ppt salinity. It is also higher in aged seawater than in crude seawater.

The effluents resulted in chronic effects on the species composition and early development of benthic marine algae growing in Si Racha Bay.

Introduction

Many surveys and research projects on marine environments with emphasis on the aquatic resources in Thai waters have been made. They point out that change of algal composition and decrease of algal biomass by water pollution occur along the coast of Si Racha Bay and Bang Pra Bay, Chonburi Province. Because there are many tapioca mills in this province, large amounts of waste products are discharged daily into the coastal waters of these bays.

This work investigated the changes in the species composition and the early development of dominant species of benthic marine algae growing on the coasts of Si Racha Bay and Bang Pra Bay with water quality and salinity as related ecological factors.

Materials and Methods

The surveys of species composition of the benthic marine algae growing on the coast of Bang Pra Bay and Si Racha Bay were done in December 1976-September 1978 and December 1982-March 1983.

The most dominant species is *Padina*, especially *P. australis* Hauck (Phaeophyta, Dictyotales) which is widely distributed in warm waters and utilized as fertilizer or feed for domestic animals in South Asian countries (Zaneveld 1955; Rao 1965; Michanek 1971). Fertile tetrasporic plants were observed for early development of *P. australis* were collected from the beach in front of the Si Racha Marine Biological Station, Kasetsart University, Si Racha, in January-March 1983. After collection they were immediately transported in a plastic container to the laboratory of the Faculty of Fisheries, Kasetsart University, Bangkok. Pieces with mature tetrasporangia were cut and rinsed several times with filtrated seawater, put in culture dishes containing filtrated seawater and set overnight under laboratory conditions.

The tetraspores liberated were pipetted on glass slides in petri dishes (90-mm diameter) containing 40 ml of different salinities of seawater. The seawater used in salinity experiments was collected near the outfall of the effluents from tapioca mills discharging into coastal waters. The salinity of this seawater was 32.2 ppt. Lower salinities, 19.5, 22.0, 26.0, 28.0 and 30.0 ppt, were obtained by diluting with distilled water. Higher salinities, 35.0, 38.0, 42.0, 44.5, 48.0 and 50.5 ppt, were obtained by mixing the seawater with seawater heated to double concentration.

The salinity in each concentration was measured with a refractometer/salinometer, American Optical Corporation, USA, with temperature compensation. The germination rate of the algae in each salinity was determined after seven days. Light intensity varied from 130 to 260 ft-c and temperature from 24°C (December) to 34°C (March).

Results and Discussion

The floral components of benthic algae which populated the coast of Bang Pra Bay and Si Racha Bay before 1974, 1976-1978 and 1982-1983 are listed in Table 1. The variations of floral components at each station of Bang Pra Bay and Si Racha Bay from December 1976 to September 1978 are listed in Table 2.

Before 1974 (Velasquez and Lewmanomont 1975), there were one blue-green alga, four green algae, 13 brown algae and 21 red algae with a total of 39 species. The herbarium specimens in 1976-1978 and 1982-1983, however, show a decrease in brown and red algae. There are obvious differences in the floral components of the station at Bang Pra Bay and at Si Racha Bay. One species of blue-green algae, *Brachyotrichia quoyi*, grows in Si Racha Bay, but not in Bang Pra Bay. The total number of species at Si Racha Bay is significantly smaller than that at Bang Pra Bay.

These results suggest that some ecological changes occurred between 1974 and 1976, which greatly affected especially the brown and red algae. In the herbarium specimens of 1976-1978 and 1982-1983, there were no *Sargassum* (brown algae) and *Laurencia obtusa* (red algae). Hirose (1977) mentioned that *Sargassum* spp. have the lowest tolerance to pollution. On the other hand, the species of *Enteromorpha flexuosa*, *Padina australis*, *Gracilaria crassa* and *Hypnea cornuta* were common and widely distributed on the beach of these bays throughout the year. *Padina australis* was observed to be most abundant indicating that these species have wide tolerance to pollution.

According to Phramsuthirak (1979), the water quality of Si Racha Bay is the same as that of the Gulf of Thailand during high tide, but that it deteriorates at low tide. He attributed the change to water pollutants in the effluents from tapioca mills which influence the water quality up to 1,000 m from the coastline during the low tide.

Water pollution exhibited by the seawater collected near the outfall of the effluents from tapioca mills influenced the early development of *P. australis*. The germination is shown in Fig. 1 and the germination rate in Fig. 2.

The liberated tetraspores were spherical with one nucleus in the center and many light brown chromatophores (Fig. 1, no. 1). The mean diameter was 63.0 ± 5.8μm (N = 175). After becoming attached to the substratum the tetraspores germinated immediately with a protuberance (Fig. 1, no. 2). This protuberance developed into a rhizoidal initial which developed further into an uniseriate rhizoid (Fig. 1, no. 3). Some germlings developed two rhizoids running in opposite directions (Fig. 1, no. 4). Deep pigmentation and protoplasm concentration occurred in some rhizoidal cells (Fig. 1, no. 5). Periclinical divisions to the axis of the rhizoid followed, and initial shoots were formed (Fig. 1, nos. 6, 7). Cell divisions occurred in the remaining upper cell which was cut off by the first segmentation, producing a rhizoidal initial. This remaining cell developed into a nodule that formed an erect shoot (Fig. 1, no. 8). The erect shoot from the nodule differentiated into two apical cells and the initial shoot of the rhizoid also developed into an erect shoot with a meristematic cell (Fig. 1, no. 9).

The germination manner of the tetraspores of *P. australis* in the seawater affected by the effluents from tapioca mills was much the same as for *P. pavonia* (Carter 1927), *P. japonica* (Nishibayashi and Inoh 1959; Umezaki and Yoneda 1962), *P. crassa* (Kumagae 1976) and *P. gymnospora* (Mshigeni and Mkwizu 1978). The significant influence of the effluents on the germination manner of tetraspores of this species has not been observed. Primary shoots were formed not only from the nodule, but also from the rhizoid. This observation suggests that the plants of *P. australis* can grow and increase in number not only by sexual reproduction, but also by vegetative reproduction, which plays a very important role in the propagation of this species throughout the year. The species of *Sargassum* and *L. obtusa* which could not be found in the two bays in 1976-1978 and 1982-1983 did not have such vegetative reproduction in their life history.

The tetraspores of *P. australis* did not germinate outside the salinity range of 19.5-50.5 ppt of the seawater collected near the outfall of the effluents from tapioca mills. The mean germination rates varied from 3.7% (at 22.0 ppt) to 31.8% (at 32.2 ppt) and they were also better in higher than in lower salinities. On the other hand, the tetraspores of this species showed better germination rates in the same seawater which was stored over two months in the dark.

The results of these salinity experiments suggest that the species of *P. australis* is capable of adaptation to a broad range of salinity variations. Such responses to salinity variations seem to reflect the development of a distinct character adapted to those environmental conditions in the tidal areas that undergo diurnal changes by tidal fluctuations and seasonal changes during the rainy or dry seasons.

Better germination in aged seawater suggests that the pollutants which affect the germination of tetraspores decompose during storage.

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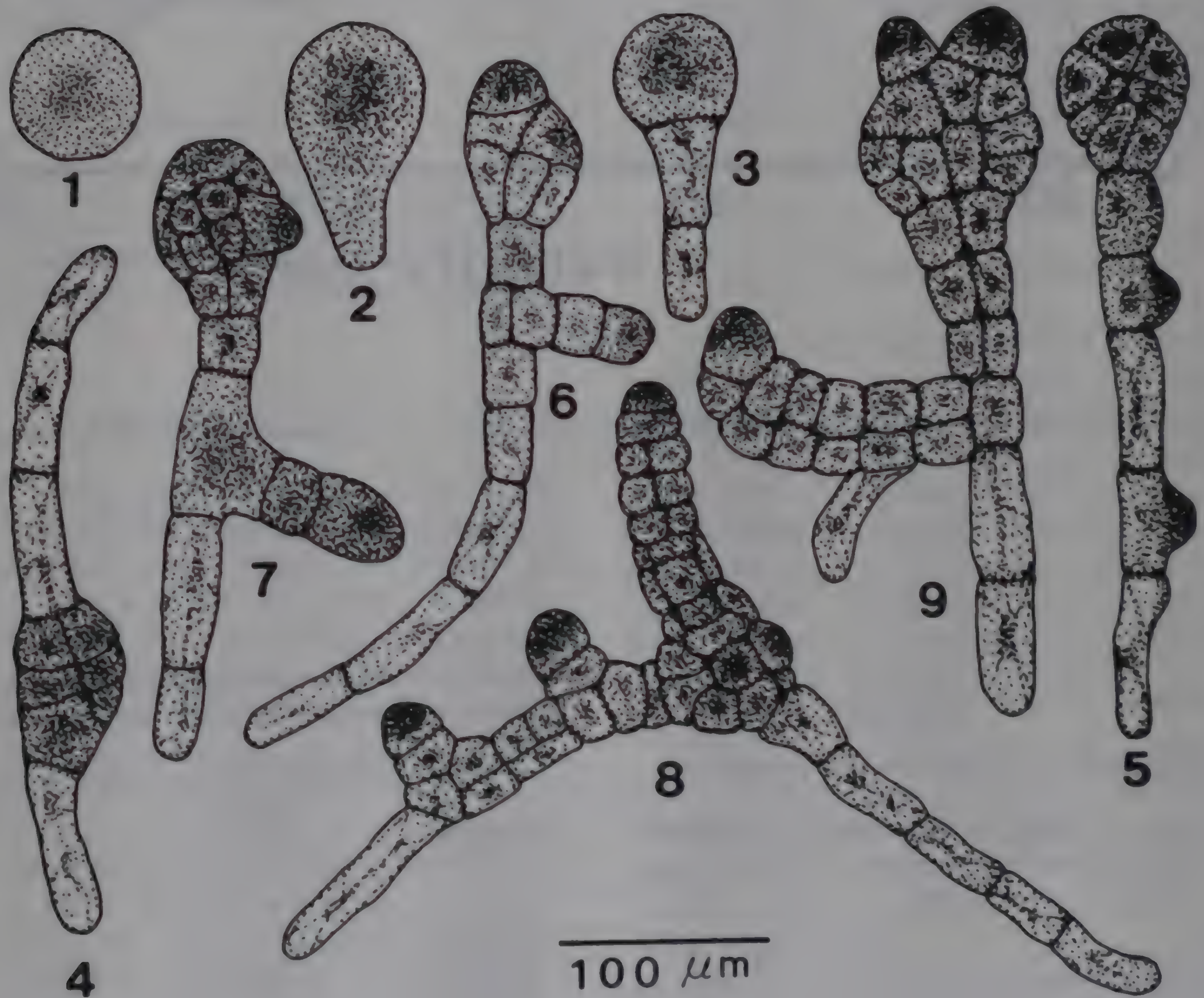

Fig. 1. Germination of the tetraspores of *Padina australis*. 1: liberated tetraspore, 2: terminal protrusion developing from the tetraspore, 3: rhizoid developing, 4: germling with two rhizoids, 5-9: further development of germlings and shoot initials are formed on germling body and rhizoidal cells.

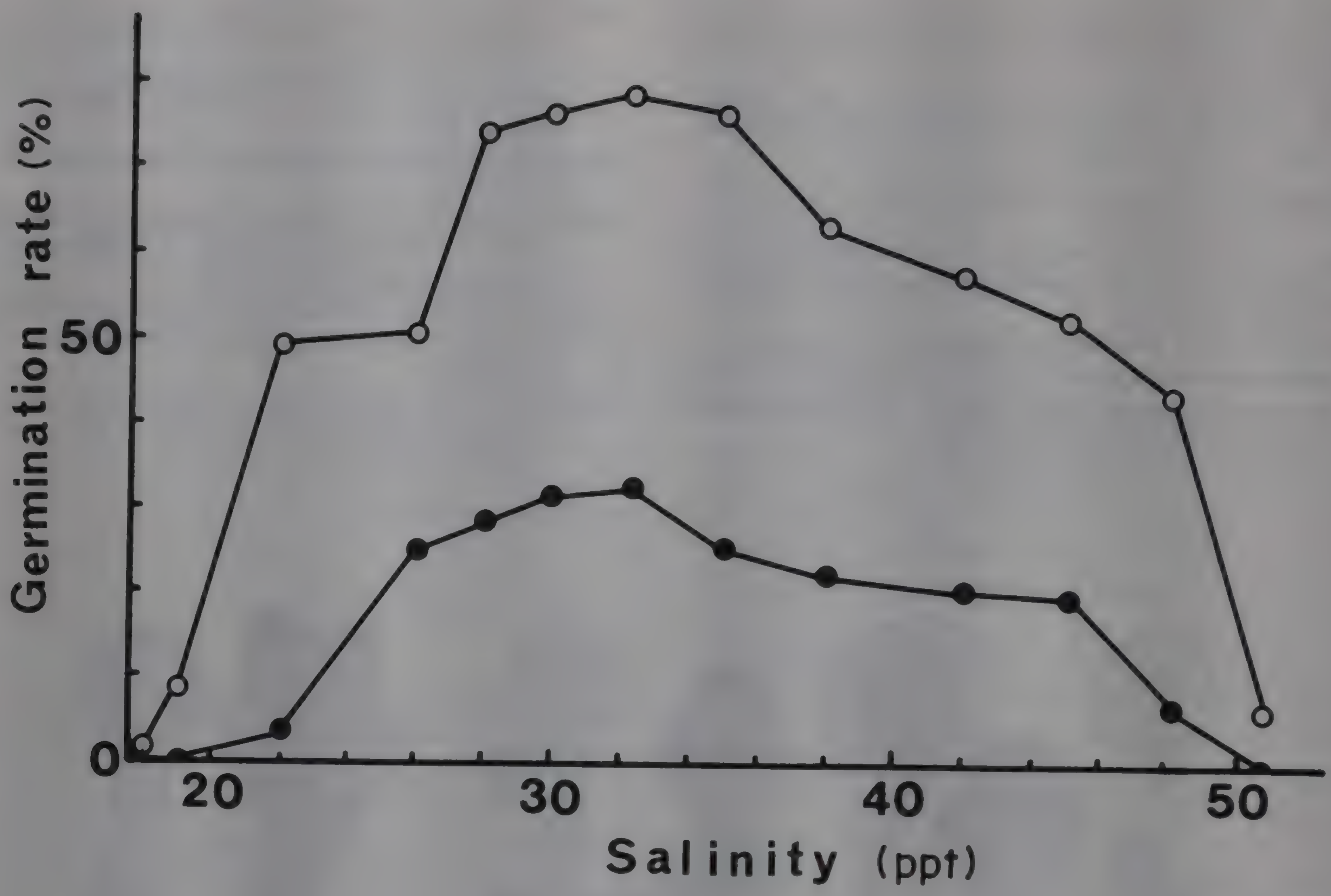

Fig. 2. Mean germination rate of the tetraspore of *Padina australis* in various salinities. ○-○: storaged seawater, ●-●: crude seawater.

Table 1. Comparison of floral components on the beach of Si Racha Bay.

Algae	Year < 1974	 1976-1978	 1982-1983
Cyanophyta	1	1	1
Chlorophyta	4	4	4
Phaeophyta	13	7	6
Rhodophyta	21	15	14
Total	39	27	25

Table 2. Comparison of floral components at each station of Bang Pra Bay and Si Racha Bay (December 1976 to September 1978).

Algae	Bang Pra		Si Racha		
	1	2	3	4	5
Cyanophyta			1	1	1
Chlorophyta	2	4	3	2	2
Phaeophyta	6	6	5	5	5
Rhodophyta	8	10	10	6	4
Total	16	20	18	13	11

Bacterial Depuration of Oyster (*Crassostrea iredalei* Faustino) in the Philippines*

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Abstract

The minimum salinity value for successful depuration of *Crassostrea iredalei* Faustino is 17.5 ppt. Lower than this, depuration is inconsistent and ineffective. The establishment of the minimum salinity value requires the adjustment of the salinity of the seawater for depuration to appropriate levels.

Introduction

Philippine oysters suffer from poor public health image in the international market. A few years ago, Singapore authorities were alerted by reports of gastrointestinal outbreaks in Singapore following consumption of oysters from the Philippines known to contain high levels of fecal coliforms, *Salmonella* and other pathogenic bacteria (Jaranilla, unpublished data; Rosario et al. 1982). As a consequence, the demand for Philippine oysters had been reduced (BFAR 1982).

Philippine oysters, usually grown in polluted marine waters, readily ingest *Escherichia coli* and other coliforms and concentrate these bacteria within their digestive system to exceptionally high levels (Fleet 1978). To overcome this predicament, the process of depuration is used. It is the natural capacity of the oysters to purify themselves of contaminating organisms through their feeding and excretory activities when placed in clean waters. Although oyster depuration has been successfully practiced in many countries, the Philippines has yet to come up with a process that is unique to both the local oyster species and to the environment.

Experiments were thus conducted to develop a depuration process suitable for the oyster *Crassostrea iredalei* Faustino and to the prevailing environmental conditions, utilizing resources provided by the Japan International Cooperation Agency.

Materials and Methods

An intake pipe directed seawater into an intake well from which it was pumped to a reservoir. Seawater from the reservoir was treated by primary and secondary filters (sand filter and cartridge filter, respectively) and then pumped into an elevated tank. From there, the filtered seawater underwent ultraviolet treatment by ultraviolet ray water sterilizer (Steritron Model SF-8 NSH) and became available in the Seafarming Research and Development Center (SRDC) Oyster Treatment Room, coded as SW3, at a flow rate of 30 l/min.

Preparation of Oysters

Oysters were harvested 16-18 hours earlier, from Lucao, Dagupan City, where they were grown by the hanging method. They were culled into the desired grade in the Oyster Treatment Room, placed in native baskets and brought to Dawel River, Dagupan City, for reconditioning for two days. After this, the oysters were brought back to SRDC and were washed with filtered seawater using a high pressure water spray to remove mud and adhering organisms and then arranged in single layers on plastic trays for depuration. The size and number of oysters per tray were noted.

The trays were suspended in fiber-reinforced plastic (FRP) depuration tanks measuring 1.79 x 0.85 x 0.54 m. They had a meshed base to permit unimpeded flow of water over the oysters and to allow excreted oyster feces to sink on the tank bottom and not in the immediate vicinity of the oysters.

SW3 was sprinkled over the oysters in a continuous system of depuration. The oysters were covered with 3- to 4-cm deep seawater in the depuration tank. Water level in the tank was kept constant by a pipe which drained water from the bottom and which was held at the same level as that in the tank.

The oysters in the depuration tanks were checked from time to time for mortality. Dead oysters were removed immediately and the number noted.

During a 48-hour depuration period, the SW3 temperature, salinity, dissolved oxygen and pH were measured every 12 hours using a temperature-salinity bridge (Hamon Model 602), dissolved oxygen meter (Yellow Springs Instruments Model 58) and pH meter (Central Kagaku Company Model UC-22).

Simultaneously, 20-25 pieces of oysters and 200-ml SW3 samples were collected for microbiological analyses. The most probable number (MPN) of total coliforms and *E. coli* of oyster samples were established according to the modified American Public Health Association (APHA) procedures described by Cook and Dicharry (unpublished data). Those of SW3 samples were determined according to APHA (1977) procedures. Standard plate counts (SPC) were made on agar medium containing 0.5% peptone, 0.5% tryptone, 0.25% yeast extract, 0.5% sodium chloride, 0.1% glucose and 2.0% agar. Plates were incubated at 35°C for 48 hours.

The experiments were carried out with coliforms in general, and *E. coli* in particular, since these microorganisms are widely used as indicators of sewage and human fecal pollution (Wood 1976). For convenience, the dimensional units for MPN and SPC are omitted in the presentation of data since levels for oysters are MPN/100 g and SPC/g and for water samples, MPN/100 ml.

Results and Discussion

The results from the 13 experiments conducted from 18 March to 24 July 1985 indicate that the oysters were capable of purifying themselves of contaminating organisms under certain salinity conditions. In Table 1, the experiments span through dry (March-May) and wet (June-July) seasons. Consequently, the effect of rainwater on depuration was observed, considering that the design of the seawater intake well allowed contamination by fresh or rainwater during very heavy rainfall. Temperature, dissolved oxygen and pH did not vary much in all the individual experiments. Overall, salinity proved to be the most crucial factor among all the water parameters monitored during the depuration experiments.

When the oysters were exposed to salinity levels of 17.5-31.1 ppt (Fig. 1), depuration was effective because the oysters (initial total coliform MPN = 8,260; initial *E. coli* MPN =4,863) were rendered microbiologically safe (total coliform MPN = 161; *E. coli* MPN = 50) within 48 hours of depuration. *E. coli* alone was down to safe levels on the 12th hour. The basis for microbiological safety is United States' 230 standard. Total coliform and *E. coli* MPN levels of oysters were drastically reduced on the first 12 hours of depuration, and then generally decreased until the 48th hour. In some cases, the MPN went up at some periods between the 12th and the 48th hour. This was most likely caused by the ingestion of coliforms released when the biodeposits in the depuration tanks were resuspended in the water (Haven et al. 1978).

Depuration was inconsistent and clearly ineffective when the salinity values were down to 9.9-14.4 ppt (Fig. 2). Initial MPN levels did not change, or even increased until the 48th hour. Large oysters (4.3-14.0 cm, Table 1) were used in these experiments; however, it is improbable that the size had any bearing at all. In the studies by Haven et al. (1978) with oysters 5- to 13-cm long, no difference in depuration rate related to size was observed.

Under natural conditions, *C. iredalei* thrive best at salinities of 17-26 ppt (PCARRD 1983). During depuration, very low water salinity causes stress to oysters. Weakened physiological activity leads to ineffective depuration and even death (Rowse and Fleet 1984). Haven et al. (1978) found that when there is a difference of 10 ppt or greater between the salinity at the site of harvest and at the depuration tank, both the rate of water transport (through the gills) and the time the oyster remains open are decreased. Species differences occur regarding salinity requirements for effective depuration. For *Crassostrea gigas* and *C. angulata*, the minimum salt concentration was 20.5 ppt, and even lower for *C. virginica* (Rowse and Fleet 1984). The minimum salinity for successful depuration by *C. iredalei* based on this study is 17.5 ppt.

It was intended that plate counts during depuration would provide a correlation of the decline of the coliform with the non-coliform bacteria. However, erratic results were yielded (Figs. 1 and 2). In some instances, the final plate counts were higher after depuration than initially. This corroborates the earlier observation that sometimes during the depuration process, the oysters might re-ingest bacteria released when the discharged oyster feces are again dissolved in water. In Souness and Fleet (1979), there is evidence that at temperatures of 25°C and above, the feces undergo chemical and microbial degradation resulting in a release and multiplication of entrapped bacteria.

With very rare exceptions, total coliform and *E. coli* MPNs of SW3 were maintained at < 2.

Early in the study, mortality of oysters during depuration was rather high at 10.6% (Table 1). This is attributed to the possible stress which the oysters must have been subjected to during harvesting and during transport. In an attempt to deal with a rather high mortality rate, only healthy oysters were used in the experiments. The oysters were brought back to the natural growing waters for reconditioning after selecting those suitable for depuration, i.e., in terms of size and species. For two days, the death of already weakened oysters was allowed. Consequently, mortality during depuration was reduced from 10.6% to 1.5-5.5%.

Throughout all the experiments, the number of oysters per tray ranged from 90 to 280 pieces (Table 1). The use of single layers for depuration was upheld to exclude the possible effects of crowding.

Minimum water salinity during the depuration is a critical factor which, if left unattended, will result in unsuccessful depuration and possible public health risks. The establishment of the minimum salinity value for *C. iredalei* requires the adjustment of the salinity of the water for depuration to appropriate levels (17.5 ppt and above).

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Table 1. Oyster size, average number of oysters per tray, mortality and mean SW3 conditions recorded during depuration experiments, 1985

Experiment no.	Date conducted	Oyster size (cm)	Average no. of oysters/tray (pcs)	Mortality (%)	Mean salinity (ppt)	Mean temperature (°C)	Mean dissolved oxygen (mg/l)	Mean pH
1	18-20 March	3.3 – 8.6	143	–	31.1	28.9	4.6	8.6
2	8-10 April	4.3 – 9.6	114	–	27.4	29.1	4.6	8.6
3	15-17 April	3.5 – 9.3	112	10.6	25.9	29.8	5.0	7.2
4	22-24 April	5.3 – 9.8	122	–	27.2	29.0	3.8	8.5
5	29 Apr-1 May	4.5 – 8.8	108	–	28.1	28.8	4.5	8.1
6	13-15 May	5.0 – 9.4	123	–	22.7	29.6	5.1	7.8
7	20-22 May	4.5 – 11.4	168	–	20.3	30.2	–	8.3
8	27-29 May	5.0 – 10.0	270	5.0	21.6	29.5	5.2	8.8
9	10-12 June	3.8 – 10.0	100	5.0	19.8	29.5	6.0	8.6
10	17-19 June	4.5 – 8.8	204	1.5	17.5	29.6	5.4	8.3
11	24-26 June	4.3 – 8.6	280	2.9	14.4	28.9	3.7	8.5
12	12-14 July	6.3 – 14.0	224	5.5	9.9	28.5	3.4	8.9
13	22-24 July	5.6 – 12.7	90	1.9	10.6	29.0	4.7	8.8

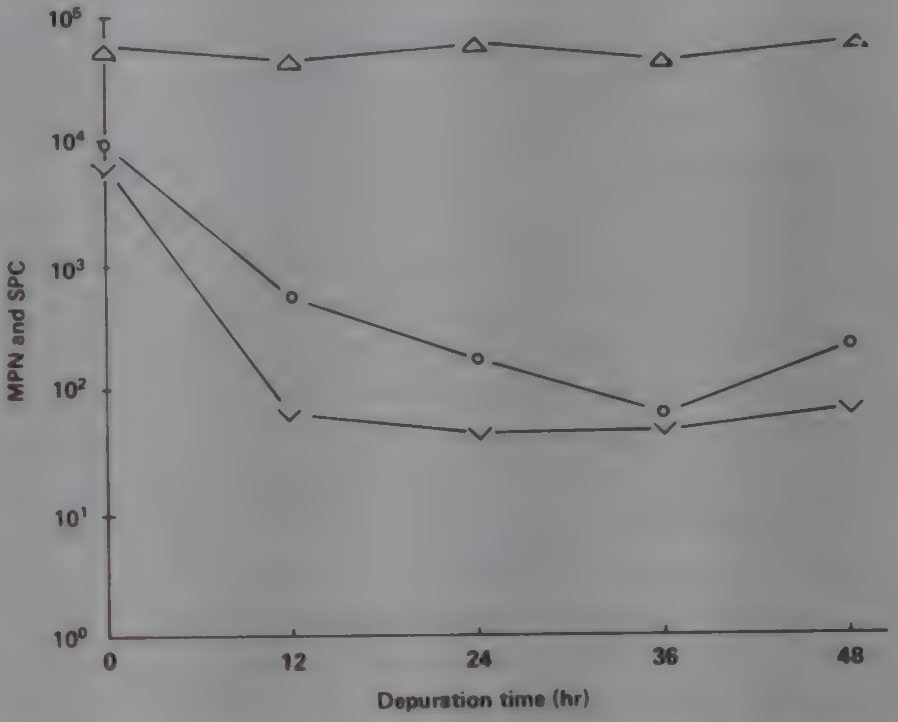

Fig. 1. Mean total coliform (○), *E. coli* (v) and standard plate counts (△) in oysters with respect to mean salinity (17.5-31.1 ppt). Experiment numbers included are 1-10. MPN = most probable number; SPC = standard plate count.

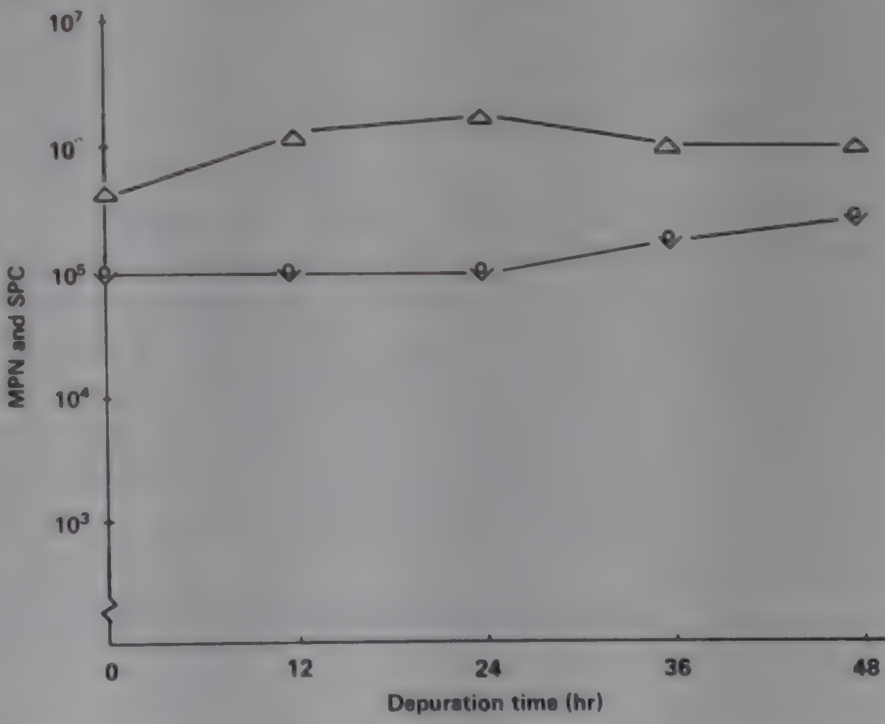

Fig. 2. Mean total coliform (○), *E. coli* (v) and standard plate counts (△) in oysters with respect to mean salinity (9.9-14.4 ppt). Experiment numbers included are 11-13.

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*Seafarming Research and Development Center Contribution No. 1

The Effects of Sodium Cyanide on Coral Reefs and Marine Fish in the Philippines

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Abstract

Sodium cyanide (NaCN) has been in use by tropical marine fish collectors in the Philippines since 1962. This paper reviews the many detrimental toxic effects of cyanide on fish which were published in the pet hobby and scientific literature. Interviews with scientists, fish collectors and aquarium industry personnel confirmed that NaCN is contributing to the destruction of Philippine coral reefs and the decline of aquarium and food fishes. About 71% of Philippine reefs are in poor to fair condition due to excessive siltation due to deforestation and the widespread use of NaCN and explosives by fishermen. There is a high mortality of fish squirted with NaCN on the reef and delayed mortalities throughout the chain of middlemen to the marine hobbyist. It is postulated that the "Sudden Death Syndrome" observed in aquaria is due to the conversion of thiocyanate in the blood back to hydrocyanic acid when the fish receives a mild stress. A program to train divers in the use of fine-mesh nets has been initiated to replace the use of NaCN. A pilot project in 1984 has demonstrated that nets can benefit the coral reefs, the collectors and the marine aquarium industry.

Tropical Marine Fish Trade

The export of marine fish from the Philippines was started by Earl Kennedy in 1957. The Philippines has the highest marine fish species diversity of all reef areas (Nybakken 1982). Of the 2,177 marine fish species found in the Philippines, about 200 species are commonly exported for the marine pet fish industry (Albaladejo and Corpuz 1981; Albaladejo, pers. comm.). No other country can match the diversity of colorful species desired by the marine aquarist. This, in conjunction with low labor costs and favorable airline shipping schedules, accounts in part for why the Philippines is the source of 75-80% of tropical marine fish sold worldwide.

Sodium cyanide (NaCN) was first used as a fish toxicant for fisheries work in Illinois (Bridges 1958). Bridges described the mode of action of cyanide as fast acting but at certain concentrations reversible. Fish which survived the initial exposure, when transferred into clean water were noted to revive with no apparent ill effect. These properties appealed to a Filipino marine fish collector named Gonzales (Robinson 1958b).

Kennedy, while he was a fish exporter in Manila, was surprised by a sudden increase in the supply of marine fish from Lubang Island, off Batangas in 1962, which he later learned was due to use of cyanide (Robinson 1985b).

The use of cyanide to collect fish spread throughout the Philippines. In the 1960s there were only three exporters in Manila (Robinson 1985a). Today there are about 35 companies which engage in cutthroat competition to export fish (Albaladejo, pers. comm.). The export of tropical marines rose from 845,000 kg in 1970 to 1.6 million kg in 1979. In 1980, exporters shipped a record 2.0 million kg of shipping boxes containing fish valued at US$3 million (Fleras 1984). Exports started declining in 1981 to 1.8 million kg, although actual sales rose to US$3.1. In 1982 exports dropped another 16% to 1.5 million kg. At present, the marine tropical fish trade has an export value of about US$10 million to the Philippine economy and a retail value of US$100 million worldwide (Robinson 1983c, 1983d). This does not include the additional revenues generated by aquarium supplies.

Some pet industry spokesmen in the United States imply that anticyanide articles in aquarium magazines contributed to the recent decline in exports of marine fish from the Philippines (Gratzek and Hatch 1985; Goldstein 1986); some aquarists did advocate such a boycott (Schlais 1980; Robinson 1984a).

Gratzek and Hatch (1985) acknowledged that the cyanide concentrations used by collectors on the reefs cannot be controlled. Affected fish may suffer mortalities as high as 75% at the point of collection (Dewey 1979). The collectors have 25-50% mortalities prior to the sale of the fish in Manila (Robinson 1984d). Exporters and wholesalers are suffering mortalities of 15-75% of their stock with 30% probably being about average (Dewey 1979). Retail outlets in the United States may suffer mortalities of 30% or more (Hemdal 1984).

Fish Collection Methods

Noyes (1976) stated that sodium cyanide also does great damage to corals and other organisms in the area of concentration. These concerns induced a British biologist, Alec R. Dawson Shepherd, to visit the Philippines.

Dawson Shepherd (1977) interviewed exporters and directly observed the collection of marine fish. The collector uses a squeeze bottle containing NaCN powder or a tablet of NaCN which dissolves as fishing continues. Dawson Shepherd (1977) estimated that 90% of the fish from the Philippines were collected with NaCN. Reports by the Philippine Bureau of Fisheries and Aquatic Resources (BFAR) confirmed the widespread use of NaCN (Sy and Corpuz 1984; Albaladejo et al. 1984).

Dawson Shepherd (1977) saw no evidence of damage to coral caused by NaCN collection; if areas of reef were locally damaged by cyanide it seems logical that the effects would not be widespread, and the reef left behind would recover. However, many good sources of aquarium fishes, particularly at the species level, are highly localized. Most species of aquarium fishes are found in collectible numbers in extremely limited areas. The areas are, in general, well known, and in most cases are already exploited. Juvenile majestic angelfish *Euxiphipops navarachus,* for example, were not found all over the Philippines but in localized areas off the Island of Cebu.

Because collectors with poison had a far higher collection success rate than those without, there was a real danger that areas of reef would be fished out of popular aquarium species (Dawson Shepherd 1977).

Albaladejo and Corpuz (1981) advocated that the use of NaCN should be stopped and its disposal regulated. Their report indicated cyanide was only used to collect certain high priced species of angelfish, triggerfish, ribbon eel and blue tang which could not be caught by nets. They advocated that BFAR develop a comprehensive resource management scheme to protect Philippine coral reefs and thus guarantee the continuous supply of marine aquarium fishes.

As Straughan (1959, 1973) noted, marine fish can be collected either with drugs or by the use of nets. Robinson (1981), a professional fish collector in the Sea of Cortez, Mexico, strongly advocated nets for collection purposes.

In Hawaii and Australia the use of drugs is banned and nets are used routinely. Siri and Barnett (1980a, 1980b, 1980c) described collection procedures in Hawaii. The diver is generally well equippped with nets, swimfins, mask, snorkle, wet suit and SCUBA gear which allows him to stay underwater at depths down to 30 m or more. Havecotte (1984) described a variety of techniques for collecting without drugs. Robinson (1983a, 1983b, 1983c, 1984h) lived and worked with collectors on Santiago Island, Pangasinan, Philippines, where the only diving equipment consisted of bamboo goggles and wooden food paddles. Almost all fish were caught with cyanide by divers using Hookah apparatus. Robinson learned that exporters sold the cyanide to collectors.

Studies of Cyanide Histopathology by Aquarists

Dempster and Donaldson (1974) found that fishes exposed to a sublethal dose of cyanide exhibited no apparent adverse effect until one or two weeks after they had been collected. Fishes were usually received from the collector one or two weeks from the time of collection with cyanide, and ordinarily were not fed during this period. An indication of liver damage appeared shortly after active feeding was resumed. Occasionally fishes with liver damage died immediately after their first large meal. However, death as a result of liver damage was generally delayed for a week or more. During this period the fishes displayed a noticeable loss of appetite and became lethargic.

They suspected that cyanide was responsible for the many unexplained deaths of newly arrived coral reef fishes from the Philippines at the Steinhart Aquarium. Examination of the dead fishes invariably showed gross liver changes. Microscopic tissue examination also showed abnormalities of the kidney, spleen and brain of the dead fishes.

Dempster and Donaldson (1974) exposed various marine fish to various doses of NaCN. On microscopic examination, both surviving and succumbed fish showed gross liver abnormalities. In every instance, most of the parenchymal liver cells had been replaced by fat. The spleens were also found to be abnormal, evidenced by spotting with iron pigment. The kidneys and brains also contained excessive amounts of iron pigment.

Bellwood (1981a) reported histological damage to the intestine of domino damselfish, *Dascyllus trimaculatus,* experimentally exposed to 1 mg/l or to 5 mg/l for 2-3 min. The observations included changes in the color of the liver and spleen and blotchy patches in the liver after 48 hours. Cyanide in the stomach resulted in sloughing of the gastric mucosa (inner lining) followed by cell degeneration. These observations would help explain the symptoms of the starvation syndrome of fish suspected of having been collected with NaCN (Herwig 1980a, 1980b).

Bellwood (1981b) exposed three specimens of *Pomacentrus violascens* to 1.84 mg/l of radioactive potassium cyanide ($K^{14}CN$) for about 2.5 min. The fish were then removed and sacrificed. The specimens were dissected and the distribution of potassium cyanide in various organs estimated by measuring the radioactivity. The tissues with the highest radioactive counts were the spleen (2,284 dpm/mg), the gills (1,187 dpm/mg), the liver (834 dpm/mg) and the brain (808 dpm/mg). This led

Bellwood to suggest that cyanide was rapidly taken up by the fish through the gills, with less taken up from the stomach (271-404 dpm/mg). The damage to the stomach and anterior intestine was attributed to the fish swallowing water containing cyanide, while the extremely high counts in the spleen and liver would be because these organs concentrate blood-borne cyanide.

Cyanide Toxicity

The controversy within the pet industry concerning cyanide has raged without reference to the scientific literature (Goldstein 1982, 1986; Edel 1982; Anon. 1985). Rubec and Pratt (1984) acknowledged that very little scientific research has been published on the effects of cyanide on marine fish. They summarized numerous papers which confirmed that cyanide is very harmful to freshwater fish. The early research found cyanide to be acutely toxic at concentrations generally greater than 0.1-0.3 mg/l causing death within 96 hours (Doudoroff 1980). Chronic toxicity also occurs when fish exposed to cyanide do not die within 96 hours, but suffer damage and stress which leads to their subsequent death (Leduc 1984). Low concentrations in the range of 0.005-0.01 mg/l of hydrocyanic acid (HCN) were found on prolonged exposure to have many adverse effects on fish eggs, fry and adult fish, such as reduced growth, impaired swimming performance, increased metabolism, inhibition of reproduction due to alteration of lipid metabolism and increased respiratory rates (Leduc 1984); damage to reproductive organs (Ruby et al. 1979); and reduction of hatching success and survival (Cheng and Ruby 1981).

Many of these effects can be traced to the fact that cyanide interferes with oxygen metabolism by blocking key enzyme systems such as cytochrome oxidase, reduces the capacity of hemoglobin to carry oxygen in the blood and blocks enzymatic pathways in the liver. Some of the effects, such as blocking enzyme function, are irreversible and hence lead to the death of the fish.

Bellwood (1981a) found that domino damselfish survived exposures at 5 mg/l of NaCN for 2-3 min. The fact that several specimens had "recovered" after 62 days, showed that the fish's intestine could regenerate after a low dose exposure.

When NaCN is dissolved in water, it dissociates to hydrocyanic acid (HCN) which is rapidly taken up by the fish. Within a few hours after exposure, an enzyme called rhodanase (thiosulfate sulfur transferase) converts the HCN to thiocyanate (SCN). Until recently thiocyanate was believed to be relatively nontoxic to the fish (Leduc 1980). It is slowly excreted in the urine.

Heming et al. (1985) observed anomalous deaths in brook trout *(Salvelinus fontinalis)* and rainbow trout exposed to SCN characterized by convulsions, gasping, loss of equilibrium and buoyancy, flaring of the opercula, darkening of the skin epithelium and, within minutes, cessation of ventilation and extreme rigor. This "Sudden Death Syndrome" (SDS) could be triggered by strenuous exercise, abrupt changes in photoperiod and increased levels of spontaneous activity. The SDS may involve a direct effect of SCN ions on neuromuscular functioning or be due to the conversion of SCN to HCN in the blood. These symptoms are similar to the observed deaths of marine fish in dealers' and aquarists' tanks (Jimenez, pers. comm.).

Dixon (pers. comm.) believed that fish exposed to a pulse dose of NaCN become very susceptible to any kind of stress. This may be because SCN forms in the blood due to action by the enzyme rhodanase on HCN. Any mild stress can cause an increase in blood pH which acts on the SCN converting it back to HCN. Dixon suspected that HCN in the blood induces the SDS. It may be fatal to the fish by acting on neurological centers in the brain.

The Net Training Program

Robinson (1984b) returned to the Philippines to participate in a program to train Filipinos in the use of nets for collecting marine fish. The Project Compassion training course sponsored by the Environmental Centre of the Philippines started in March 1984 (Robinson 1984c, 1984d). The course gave three days of orientation and theory and seven days of practical skills training. The training also included economic, social and environmental arguments for better reef fisheries management (Robinson 1984c, 1984d).

Robinson (1984e, 1984f) summarized the success of the education and training of the first two classes of net fishermen from Santiago Island. A third training course was given near Tagbilaran, on the Island of Bohol. This course also succeeded in convincing some very hard core cyaniders that nets were a better way to collect fish. The three projects trained 30 cyanide users, plus members of their families, in net collection procedures during 1984. These persons indicated they would be willing to train others.

Tropical marines which were certified net-caught were shipped from the Philippines in April and May (DeBernardo 1984; Smith 1984). These fish were shipped to Guaranteed Hawaiian in Pearl City, Hawaii. Fish were exported to demonstrate not ony that fish could be collected with nets, but also that this could be done as economically as with fish caught by cyanide.

According to John Johnson of Guaranteed Hawaiian, the survival of cyanided fish collected in the villages was about 30%, while the survival of net caught fish into

Manila and being exported was approximately 98% (Smith 1984). During 1984, marine fish caught with nets were exported with about 95% survival (Robinson 1984g).

Commercial Inshore Fishery

Robinson (1983b, 1983c) observed that NaCN was used extensively for capturing food fish. The cyanide fishermen with a "production-at-all-costs" mentality were noted to impoverish the non-collecting members of their own community (Robinson 1984b). The cyanider makes his living by raiding and damaging other people's fishing grounds as well.

The Central Visayas (Philippines) Resource Management Project I (CVRMP) issued a report in 1984, which noted that there was widespread damage to the reefs due to dynamite fishing, sodium cyanide collecting as well as other destructive forms of fishing. Many reefs were barely recruiting fishes.

Harmful Effects of Cyanide on Humans

Cyanide is extremely hazardous to humans when it is inhaled, ingested or absorbed across the skin (Way 1981). There are reports that the deaths of several people from villages in Bolinao, Pangasinan, have been attributed to cyanide poisoning (Albaladejo and Corpuz 1981). Eating a steady diet of cyanide-poisoned fish does not appear harmful to adults but may harm children (Robinson 1983a, 1983b, 1984e). Deaths from cyanide poisoning have occurred when fishermen have brought back food fish in the plastic bags used to carry NaCN tablets or when they ate fish which had cyanide poisoned baits still in their stomachs (Carolino, pers. comm.).

Effects of Cyanide on Coral Reefs

The Danajon Bank, in the Bohol Strait, between the islands of Bohol and Cebu until recently had fed the people of Bohol and Cebu for centuries. The decline of the Danajon Bank began in 1950s with heavy dynamite usage (McLarney 1986). Yet in the 1960s when the cyanide method began, the Danajon still represented a bonanza for the entire Philippine aquarium fish trade. Aquarium-sized fish tend to remain if there is enough living coral.

Robinson (1984c) noted that each cyanide collector squirts about 50 coral heads per day and dives about 225 days per year. He estimated that there were 600 full-time cyanide collectors and about 400 seasonal cyaniders. A simple extrapolation indicates that 1,000 collectors cover about 11 million coral heads per year. While it is not known what the rate of mortality of coral heads is from being squirted by cyanide, the figures give some idea of the potential magnitude of the problem.

Robinson (pers. comm.) maintained that it is possible to distinguish between coral heads which have been dynamited and those which have been affected by cyanide. Coral heads exposed to cyanide are usually dead, but intact, while corals which have been dynamited are fragmented. On the other hand, Ferraris (pers. comm.) believed that cyanide has little impact on coral. He worked for the University of the Philippines on the Gomez et al. (1981) reef survey and conducted a survey of the fishes of Bolinao (Pangasinan Province) from 1976 to 1980. He squirted corals with cyanide and did not observe coral mortality on his return, days and several weeks later.

The Coral Reef Research Team with BFAR has investigated the effects of squirting NaCN onto coral reefs in 1979 and 1980 (Miclat, pers. comm.). In the first study (1979), one dose of cyanide was given. Sceleratinian corals *(Acropora, Pocilopora, Porites)* reacted by retracting their polyps and exuding a mucoid substance. In a manner of minutes the polyps came out again positioned in the usual way. Octocoralion soft corals *(Xenia, Anthelia, Sarcophyton* and *Lobophyton)* retracted their polyps but were not observed to exude mucus. These species of soft and stony corals were observed feeding with their polyps extended 1-2 days later.

In a second study (1980), a second dose of cyanide was given four months after the first. A day after the second application all corals appeared to have recovered. But when the stations were revisited three months later, all corals in the test cyanide quadrats were dead while those in the control quadrats remained alive, except for 25% of the branching corals in one quadrat which showed signs of grazing by crown of thorns starfish *(Acanthaster* sp.*)*. The cyanide exposed corals were distinct from the rest of the coral heads in the immediate vicinity being uniformly encrusted by brown-colored algae. While this study was marred by the *Acanthaster* predation, it appeared to indicate that NaCN kills corals. Former cyanide collectors also stated that coral heads exposed to cyanide die one week to one month after exposure (Carolino, pers. comm.). Initially, the coral heads take on a bleached white appearance and later become encrusted with algae.

The International Marinelife Alliance

In June 1985, a non-profit organization, the International Marinelife Alliance (IMA) was formed to conserve the diversity of sea life, protect marine environments and promote sustainable use of marine resources. Unlike other conservation groups, it seeks protection for ecosystems rather than individual species.

The IMA is advocating a program to train divers in the use of nets for collecting marine fish and scientific research to test for cyanide residues in fish and define the toxicity of cyanide on marine fish, corals and crustaceans. A reliable test for thiocyanate in the blood of marine fish would enable the Philippine government and regulatory agencies of other countries importing marine fish to check whether the fish were caught with cyanide.

The net training proposal of Robinson (1984c, 1984d) would work with nucleus of 6-10 Filipino collectors who received training in 1984. They would conduct village-based courses to train the 1,000 cyanide fishermen in net collection procedures. The program would take 1-2 years to complete.

Scientific organizations such as the European Ichthyological Union and the American Society of Ichthyologists and Herpetologists have passed resolutions against the use of NaCN and other destructive fishing methods which are destroying coral reefs. Concern has also been expressed by the Federation of American Aquarium Societies, Windows To The Sea Aquarium Society and Ornamental Fish International.

In June 1986, the Philippine Minister for Agriculture and Food pledged the support of his government, BFAR and regulatory agencies in publicizing and implementing the IMA's programs of net training and research. He authorized the IMA to seek funds on behalf of the Philippine government for these programs.

On 25 August 1986, Fisheries Administrative Order No. 155 was issued, regulating the use of fine-meshed nets in fishing. The proclamation legalized the use of fine-meshed nets (< 30 mm) for the capture of marine aquarium/ornamental fish plus other fish and crustaceans important for aquaculture.

A number of international agencies concerned with marine resources and social development have expressed interest in supporting the net training proposal of Robinson (1985c, 1985d). Philippine scientists at the University of the Philippines and Silliman University are interested in joint scientific research with Canadian and American scientists to evaluate the effects of cyanide on coral reefs and on tropical marine fish.

In October 1986, a joint agreement was obtained between the Ministry of Agriculture and Food, IMA and the Philippine Tropical Fish Exporters Association, Inc., to jointly sponsor and help finance a countrywide net training program. A pilot project in Pagbilao, Quezon, in November 1986 would be run with personnel from the Bureau of Agriculture Extension, Bureau of Fisheries and Aquatic Resources, Philippine Fish Development Authority, International Marinelife Alliance, local coordinators and divers from Santiago Island. All parties agreed that they would work together to help protect and conserve the coral reef environment for the benefit of the Philippines and village fishermen.

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The Toxicity of Chemical Spilt-Oil Cleaners in Japan

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Abstract

Several chemical counteragents are used to prevent and to lessen the damage to fisheries resources and marine farms by oil in Japan. These chemicals are chiefly classified into six categories according to their action modes: as emulsifying dispersants and gelatinizing agents legally permitted for application in the sea and as de- and anti-emulsifiers that might be permitted in the near future. The chemical counteragents permitted for use have low toxicity, and all of their 24-hour LA_{50} for *Oryzias latipes*, a Japanese killifish, are 3,000 mg/l and more; simultaneously, all of their toxicity on *Skeletonema costatum*, a marine centric diatom, is 100 mg/l and more. The other counteragents such as self-mixing dispersants, chemical oil fences and surface dispersants are so highly toxic that they are not yet permitted to be used in Japan.

Classification and Properties of Chemical Spilt-Oil Cleaners

Emulsifying dispersants are the most popular agents in Japan and their constituents are one or more kinds of nonionic surfactants, 20-30% in a solvent (70-80%) which is usually a mixture of biodegradable hydrocarbons such as paraffin or rarely water. When they are poured onto an oil slick on the sea and agitated by propellers of a boat or by water jets through a nozzle, oil is emulsified and dispersed into the water column as small droplets, which then undergo microbial decomposition.

Self-mixing dispersants are a kind of emulsifier, but do not need mixing operation because they contain amphiphilic substances as solvent. When sprayed on an oil slick, these agents themselves mix with the spilt oil and disperse automatically the oil as minute droplets into the water column. Owing to their dissolving properties, they are usually applied on an oil slick over a wide area by an airplane.

Chemical oil fences have strong surface-dispersing activity, and are capable of reducing an area of an oil slick when applied on the surrounding area of an oil slick. Their chemical compositions are not disclosed.

Surface dispersants can reduce surface tension of oil and disperse an oil slick into an invisible thin film, if applied directly on an oil slick. They are a kind of surfactant but their constituents are not disclosed.

Gelatinizing agents consist of a solvent and a solute which is either an oil-soluble derivative of an amino acid or some other organic substances. They solidify by themselves when in contact with water. The spilt oil is trapped into gel structures of the agents, kept afloat on the water surface, and can thus be collected easily with a fishing net or a skimmer.

The oil spilt on the sea easily absorbs the seawater and forms a highly viscous water-in-oil emulsion called oily mousse composed of 60-80% water. *De-emulsifiers* are the agents that break such an emulsion into oil and water again and *anti-emulsifiers* are the chemicals that prevent the formation of a water-in-oil emulsion by being preliminarily added to oil. Those compounds contain surfactants and a solvent.

Among the chemical counteragents mentioned above, emulsifying dispersants and gelatinizing agents are already permitted legally for use in Japan and de- and anti-emulsifiers might be legally permitted in the near future.

Test Organisms for Oil Cleaners

The toxicity test of environmental pollutants should be carried out on species of both animals and plants; it is indispensable for a test organism to be available anytime. A Japanese killifish, *Oryzias latipes*, a freshwater species commercially reared all year-round but easily acclimated to seawater, has been used as a test for some pollutants for a few decades in Japan (Japanese Industrial Standard 1971), and is accepted also for the toxicity test of oil cleaners (Japan Association for Preventing Marine Accidents 1975). But there has been no plant species standardized for the toxicity test in Japan. Being easily maintained in a laboratory, microalgae or phytoplankton are more suitable than seaweeds.

Marine green algae such as *Chlorella* sp. and *Chlamydomonas* sp. and marine diatoms such as *Nitzschia closterium* and *Skeletonema costatum* were preliminarily checked for their sensitivities to a surfactant and *S. costatum* was found the most sensitive. But its sensitivity to the surfactant was different from those of its clones, and a clone from the coastal waters at Kushiro in Hokkaido had less sensitivity. Consequently, Kushiro clone of *S. costatum* is accepted as the test organism among plant species (Japan Association for Preventing Marine Accidents 1975; Tokuda 1981).

Toxicity of Emulsifying Dispersants

The results of toxicity tests of 14 chemicals on *O. latipes* and *S. costatum* are shown in Tables 1 and 2 (Japan Association for Preventing Marine Accidents 1975; Tokuda 1981). In Table 1, 24-hour LA_{50} means the added amount of the dispersants killing 50% of the fish exposed to a test liquid for 24 hours. Although the symbol LC, which means a lethal concentration, is ordinarily used in a toxicity test, the agents tested here are scarcely soluble but colloidal in water and, therefore, the amounts of the agents in a test liquid cannot be expressed as concentration.

Only the products with 24-hour LA_{50} of 3,000 mg/l and more for *O. latipes* and which simultaneously kill *S. costatum* at the added amount of 100 mg/l and more are legally permitted in Japan, and 44 products are approved by the Marine Safety Agency, Ministry of Transportation of Japan.

Toxicity of Gelatinizing Agents

The toxicity of two gelatinizing agents is shown in Tables 3 and 4, in which A and B indicate the test liquids containing gel structures formed upon contact with water, and Af and Bf indicate the filtrates of A and B, respectively (Tokuda 1979). The permissible toxicity limits of the gelatinizing agents to the test organisms are legally set up as the same ones with the emulsifying dispersants. Three products of gelatinizing agents are approved in Japan.

Toxicity of De- and Anti-Emulsifiers

As shown in Tables 5 and 6, the toxicities of de- and anti-emulsifiers are low like those of the emulsifying dispersants and geratinizing agents. Accordingly, they would be legally permitted to be applied in Japan in the near future.

The other chemical counteragents against oil pollution are more toxic than the agents mentioned above. Consequently, they are not yet permitted to be used in Japan. Several kinds of crude oils and oil products have been studied for their toxicities and exhibit higher toxicities than the chemicals described.

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Table 1. The amounts of 14 products of emulsifying dispersants killing 50% of *Oryzias latipes* in 24 hours (24-hrLA_{50}) added respectively to the seawater.

Product No.	24-hrLA_{50} (mg/l)
1	24,000 <
2	24,000 <
3	24,000 <
4	24,000 <
5	13,000
6	24,000 <
7	24,000 <
8	24,000 <
9	24,000 <
10	17,000
11	24,000 <
12	24,000 <
13	24,000 <
14	24,000 <

N.B.: Temperature 20°C, not aerated.

Table 2. Growth of *Skeletonema costatum* in seawater containing 14 products of emulsifying dispersants at various amounts (20.0° ± 0.5°C, 4.5 klux, 14-day culture).

Product	Amount added (mg/l)								
No.	100	180	320	560	1,000	1,800	3,200	5,600	10,000
1	+	+	±	–	–	–	–	–	–
2	+	+	+	+	+	+	±	–	–
3	+	+	±	–	–	–	–	–	–
4	+	+	+	±	–	–	–	–	–
5	+	+	–	–	–	–	–	–	–
6	–	–	–	–	–	–	–	–	–
7	+	+	+	+	+	+	±	±	–
8	+	+	±	–	–	–	–	–	–
9	+	+	+	+	+	+	+	+	+
10	+	+	+	±	–	–	–	–	–
11	+	+	+	+	+	+	+	+	+
12	+	±	–	–	–	–	–	–	–
13	+	±	–	–	–	–	–	–	–
14	+	+	+	+	+	+	±	–	–

N.B.: + no effect; ± no mortality but little or no growth; – culture killed.

Table 3. The amounts of two products of gelatinizing agents killing 50% of *Oryzias latipes* in 24 and 48 hr (24-hrLA_{50} and 48-hrLA_{50}).

Test liquid	24-hrLA_{50}	48-hrLA_{50} (mg/l)
A	7,400	7,400
Af*	42,000	41,000
B	4,700	3,900
Bf*	7,100	6,800

N.B.: Temperature 20°C, not aerated.

*Af and Bf indicate the filtrates of A and B respectively.

Table 4. Growth of *Skeletonema costatum* in seawater containing two products of gelatinizing agents or their filtrates at various amounts (20.0° ± 0.5°C, 4.0 klux, 14-day culture).

Amount added	Product tested			
(mg/l)	A	Af	B	Bf
100	+	+	+	+
180	+	+	+	+
320	+	+	+	+
560	+	+	±	+
1,000	±	+	–	+
1,800	–	+	–	±
3,200	–	+	–	–
5,600	–	±	–	–
10,000	–	–	–	–

Symbols as in Table 2.

Table 5. Amounts of six trial products of de- and anti-emulsifiers killing 50% of *Oryzias latipes* in 24 and 48 hr (24-hrLA_{50} and 48-hrLA_{50}).

Trial product	24-hrLA_{50}	48-hrLA_{50} (mg/l)
1	24,500	21,300
2	32,000 <	32,000 <
3	24,000	21,300
4	32,000 <	32,000 <
5	32,000 <	29,000
6	32,000 <	32,000 <

N.B.: Temperature 20°C, not aerated.

Table 6. Growth of *Skeletonema costatum* in seawater containing six trial products of de- and anti-emulsifiers at various amounts (20.0° ±0.5°C, 4.6 klux, 14-day culture).

Amount added	No. of trial products					
(mg/l)	1	2	3	4	5	6
32	+	+	+	+	+	+
56	+	+	+	+	+	+
100	+	+	+	±	+	+
180	+	+	+	–	+	+
560	+	+	+	–	+	+
1,000	+	+	+	–	+	+
1,800	+	+	+	–	+	+
3,200	+	+	±	–	+	–
5,600	+	+	–	–	+	–
10,000	–	+	–	–	+	–

Symbols as in Table 2.

Toxic Dinoflagellates in Japan

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Abstract

Several toxic dinoflagellates which differ in habitat, life cycle and distribution have been found in Japan.

Protogonyaulax tamarensis and *P. catanella* are widely distributed and produce paralytic poisons which pose a serious problem in the aquaculture of scallop and oyster. They form resting cysts which play an important role as seed populations for annual blooms and geographical dispersal. *Dinophysis fortii* and some other *Dinophysis* species produce toxins which cause diarrhetic shellfish poisoning. *Gambierdiscus toxicus*, the causative organism of ciguatera fish poisoning, is found in sand or on seaweed which grows on coral reef. Other toxic species which occur together with *G. toxicus* are *Prorocentrum lima, P. mexicanum, P. concavum, Amphidinium carterae, A. klebsii, Ostreopsis ovata* and *O. siamensis*. Some of them are common in the southern part of Japan, particularly the Ryukyu Islands.

Mass mortality of marine organisms sometimes occurs along with the red tide of *Gymnodinium nagasakiense*. The ecology of the species is obscure. Resting cysts have never been found. Blooming mechanisms of some other *Gymnodinium* and *Cochlodinium* species, which are suspected to be toxic, are still unknown.

Introduction

More than ten dinoflagellate species in several genera are responsible for toxicity and/or mass mortality of marine organisms. Their economic impacts to the fishery industry have become more severe as aquaculture of fish and shellfish is promoted extensively all around the Japanese coast.

In northern Japan scallop culture receives serious damage (Koganezawa and Kotani 1985) from paralytic toxin by *Protogonyaulax* and diarrhetic toxin by *Dinophysis*, because trading of the scallops is stopped until the levels of toxin fall lower than certain permitted level. In southern Japan, *Gambierdiscus* and some other toxic dinoflagellates, which are common in tropical ciguatera-endemic area, were found (Yasumoto et al. 1980a; Fukuyo 1981). Fortunately ciguatera is very rare in Japan, but those findings imply the possibility of occurrence of the poisoning.

Mass mortality of marine organisms is sometimes associated with the red tide of unarmored dinoflagellates, such as *Gymnodinium* and *Cochlodinium*, although the toxic substances have been unclear so far.

Research on biology of the toxic dinoflagellates and chemistry of toxins have progressed quickly in the last decade (see Steidinger and Baden 1984). The purpose of the present paper is to describe and update their taxonomy, morphology and ecology.

Species Responsible for Paralytic Shellfish Poisoning

The genus *Protogonyaulax* contains some toxic species causing paralytic shellfish poisoning. Loeblich and Loeblich (1979) use *Gessnerium*, and Balech (1985) adopts *Alexandrium* instead of *Protogonyaulax* for the genus name. Fukuyo et al. (1985) identified six species in Japan using several taxonomical criteria, such as cell shape, chain-forming ability and some thecal plate characters. Among the six species, *P. tamarensis* (Fig. 1) and *P. catanella* (Fig. 2) are toxic (Oshima and Yasumoto 1985), whereas *P. affinis* (Oshima et al. 1982) and *P. fratercula* (Noguchi et al. 1985) are nontoxic. Two other species, *P. peruviana* and *P. compressa*, have not yet been studied for toxicity.

Protogonyaulax tamarensis is round and usually slightly longer than wide. The cingulum is equatorial, descending one cingular width. The sulcus is depressed and widened posteriorly. The apical pore plate is narrow, triangular to rectangular, and has a fishhook-shaped apical pore. A ventral pore is present on the suture between the apical 1' and 4'. Cells make a chain of two or four cells. After sexual conjugation a resting cyst (Fig. 3) is formed (Anderson and Wall 1978). The cyst is ovoidal to elongate cylindrical with rounded ends. Mucilaginous substances excreted from the cyst cover the whole cyst surface. The species is widely distributed, mainly in northern Japan. No red tide has been recorded and the cell concentrations are usually less than 100 cells/l (Fukuyo 1985).

Protogonyaulax catenella is round and usually slightly wider than long. Differences from *P. tamarensis* are so minor that it is impossible to identify the two species without dissection of the thecal plates. The apical pore plate of *P. catanella* is triangular. The ventral pore is lacking. The species is also widely distributed, mainly in western Japan. Some cases of red tide have been recorded (Fukuyo 1985).

Species Responsible for Diarrhetic Shellfish Poisoning

Dinophysis fortii (Fig. 4) and *D. tripos* (Fig. 5) are confirmed as causative organisms of toxicity of shellfish by diarrhetic poison (Yasumoto et al. 1980b; Igarashi 1985). Yasumoto et al. (1985) found the toxic substances from *Dinophysis acuminata* (Fig. 6) collected in northern Japan. However from the same species collected at Tokyo Bay, Dr. M. Kodama (Kitasato Univ., pers. comm.) could not detect the toxin. Such difference of the toxicity seems to be due to variations of strains in a single species, and the similar phenomenon is known in *P. tamarensis* and *P. catanella* (Schmidt and Loeblich 1979; Oshima et al. 1982).

Dinophysis fortii is ovoid to ellipsoid in shape, deepest behind the center and compressed laterally. It is transported by the Tsushima Warm Current and blooms at up to 10,000 cells/l in several coastal areas of northern Japan where the Current mixes with nutrient rich nearshore waters.

Dinophysis tripos is large and has a peculiar shape. In hypotheca it has two projections, short dorsal and long ventral. Some taxonomists consider that this species is a synonym or one of the varieties of *D. caudata*, which is widely distributed and rarely makes red tide.

Species Responsible for Ciguatera

Gambierdiscus toxicus (Fig. 7) is the causative organism of ciguatera fish poisoning (Yasumoto et al. 1977; Adachi and Fukyo 1979). It is round to ellipsoidal in apical view, strongly compressed anterio-posteriorly. Thecal plates are densely porous. The protoplast is yellow-brown and contains a large transparent pusule ventrally. Fukuyo (1981) found it from the Ryukyu Islands, and Hara and Horiguchi (1982) from Izu Peninsula.

Associated with *Gambierdiscus toxicus* are some species belonging to *Prorocentrum, Amphidinium, Ostreopsis* and *Coolia* (Fukuyo 1981; Hara and Horiguchi 1982). Some of those species are known to produce toxic substances which may have a relationship to ciguatera (Yasumoto et al. 1980a).

In the genus *Prorocentrum, P. lima* (Fig. 8), *P. concavum* (Fig. 9) and *P. mexicanum* (Fig. 10) are toxic. *P. lima* is ovoid, widest behind the middle and compressed laterally. The anterior margin is flat or slightly concave without spine. Valves, which cover the cell surface, have many trichocyst pores lined up inside the margin and scattered over the middle. *P. concavum* is broadly ovoid. The anterior margin is concave. Valves have fine depressions and many trichocyst pores all over the surface. This species closely resembles *P. lima* in cell shape, but is distinguishable by the presence of clear fine depressions of the valves. *P. mexicanum*, which is a senior synonym of *P. rhathymum* by Loeblich et al. (1979), is oval in valve view. A short spine is clearly observable near an anterior concavity of flagellar insertion. Valves have many trichocyst pores, most of which lie in rows radially from the center and perpendicularly to the valve margin.

In the genus *Amphidinium, A. klebsii* (Fig. 11) and *A. carterae* (Fig. 12) are known to be toxic. The latter species is smaller and rounder than the former. *A. klebsii* is ellipsoidal to quadrangular in ventral view, and narrowly ellipsoidal in lateral view. The epicone is very small and crescent-shaped. It is bent to the left in ventral view.

Ostreopsis ovata (Fig. 13), *O. siamensis* (Fig. 14) and *Coolia monotis* (Fig. 15) are also toxic. *Ostreopsis ovata* is ovoidal, and *O. siamensis* is broadly ovoidal to ellipsoidal, pointed ventrally in apical view. They are compressed anterio-posteriorly in lateral view. *Coolia monotis* is small and lens-shaped. The apex goes back and the antapex is close to the ventral area. The three species have the same epithecal plate arrangement. The apical pore plate is eccentric, near the left dorsal margin, and has a slit-like pore.

Species Associated With Mass Mortality

Mass mortality of marine organisms sometimes occurs along with a red tide of some unarmored dinoflagellates. *Gymnodinium nagasakiense* (Fig. 16), which was called *Gymnodinium* sp. type '65, is one of the most harmful species. The first case of red tide with serious damage to cultured pearl oysters occurred in 1965 at Omura Bay in western Japan. After that outbreak, mass mortality has not recurred, even though the red tide reappeared (Iizuka 1976, 1979). But in 1984 and 1985, the red tide appeared widely along the east coast of Kii Peninsula and Seto Inland Sea, respectively, and caused serious economic damage to fish and shellfish fisheries. Morphology of the species was studied in detail by

Takayama and Adachi (1984). The epicone is round to triangular, and the hypocone is oval to subquadrangular. The girdle is located slightly anterior to the middle and is displaced 1/9 to 1/4 of the cell length. The sulcus runs from the epicone to the posterior end of cell. The cell has an apical groove which runs across near the apex (Takayama 1981). The species has morphological similarity to *Gyrodinium aureolum*, which is known as a toxic species in northern Europe (Tangen 1977).

Gymnodinium sp. type '84K and *Cochlodinium* sp. type '78 Yatsushiro are also toxic (Onoue et al. 1985a, 1985b). The former species formed a red tide at Kagoshima Bay. The epicone is hemispherical and has an apical lobe at the apex. The girdle is wide. The sulcus is shallow and is restricted in the hypocone. The latter species makes chains of 8 to 16 cells. In a single swimming cell the epicone is subconical, and the hypocone has prominent lobes. The girdle goes around the cell twice.

Red tides of *Noctiluca miliaris*, one of the most common dinoflagellates, are seldom associated with a fish kill. Okaichi and Nishio (1976) reported that the mortality is caused by ammonia accumulated inside the cell.

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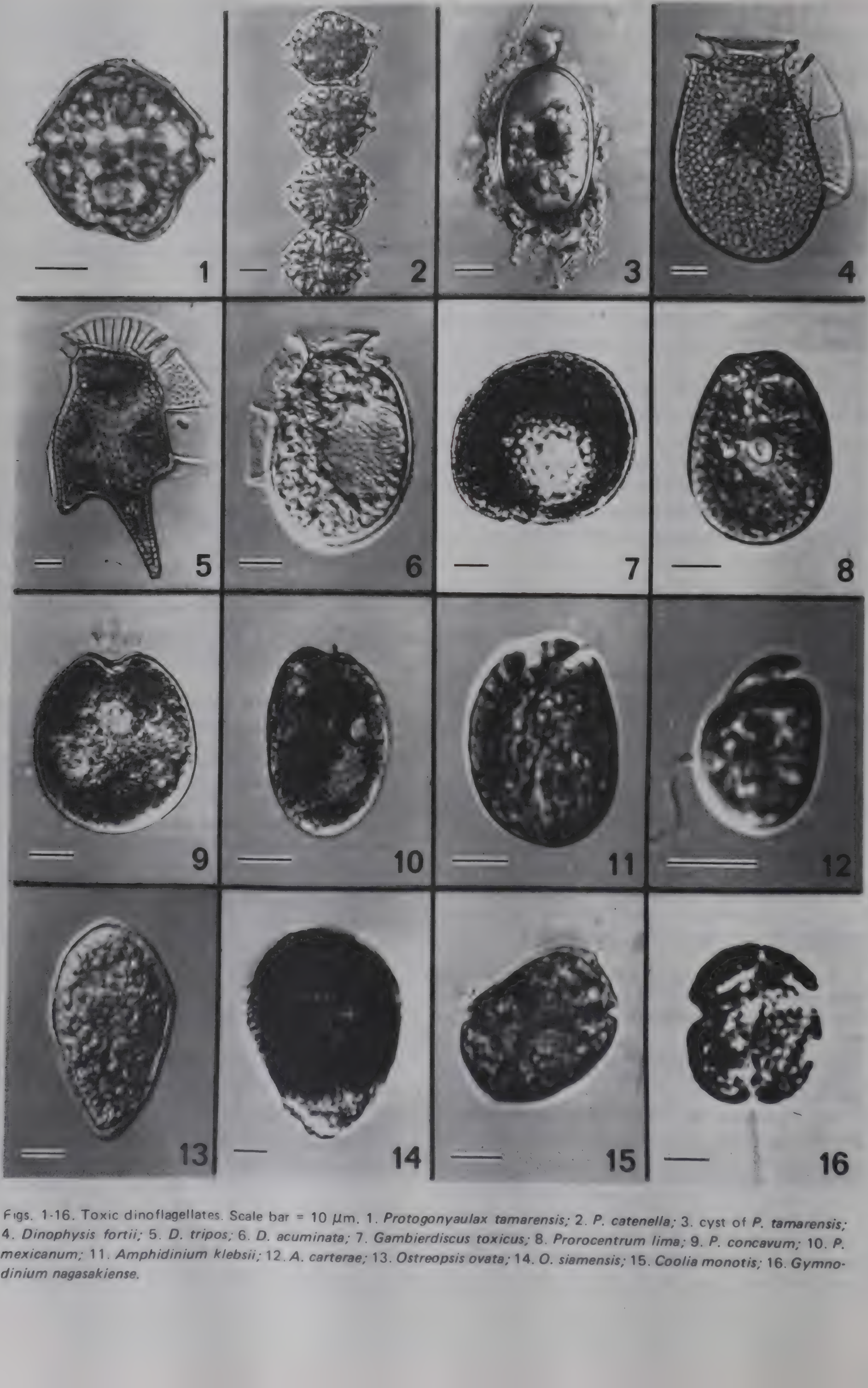

Figs. 1-16. Toxic dinoflagellates. Scale bar = 10 µm. 1. *Protogonyaulax tamarensis;* 2. *P. catenella;* 3. cyst of *P. tamarensis;* 4. *Dinophysis fortii;* 5. *D. tripos;* 6. *D. acuminata;* 7. *Gambierdiscus toxicus;* 8. *Prorocentrum lima;* 9. *P. concavum;* 10. *P. mexicanum;* 11. *Amphidinium klebsii;* 12. *A. carterae;* 13. *Ostreopsis ovata;* 14. *O. siamensis;* 15. *Coolia monotis;* 16. *Gymnodinium nagasakiense.*

Effects of Coconut Milk and Brown Sugar on Crude Toxins from Mussels Exposed to *Pyrodinium bahamense* var. *compressa*

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Abstract

During a red tide episode caused by *Pyrodinium bahamense* var. *compressa* in Western Samar, Philippines in 1983, those who were taken ill after ingesting the green mussel, *Perna viridis*, resorted to drinking coconut milk (*gata*, Pilipino) with brown sugar or unpurified sugar lumps (*tagapulot*, Pilipino) as a temporary palliative, pending medical attention. Many victims felt relief after the drink. Crude toxins (CT) were extracted from *P. viridis* exposed to *Pyrodinium* using 0.1 N HCl and reacted with either or both 5% coconut milk (CM) and 5% brown sugar (BS) for an hour. The CT, CM, BS, CT + CM, CT + BS, and CT + CM + BS were assayed in duplicates for saxitoxin using the standard mouse toxicity test. CT with initial toxicity of 2,114 MU/100 g meat was substantially detoxified after a one-hour reaction. In CT + CM, the toxicity was 664 MU/100 g; in combined CT + CM + BS the toxicity was 1,005 MU/100 g. In medium- (436-563 MU/100 g) and low-toxicity extracts (160-231 MU/100 g) no deaths in mice were recorded within one hour of injection.

Introduction

On the third week of July 1983, red tide occurred in Maqueda and Villareal Bays, Western Samar and in Leyte, Philippines (Hermes 1983; Estudillo 1983; Hermes and Villoso 1983; Estudillo and Gonzales 1984). The causative dinoflagellate, *Pyrodinium bahamense* var. *compressa* (Steidinger et al. 1980) is the same organism that caused a series of blooms during the 1970s in Papua New Guinea (Maclean 1973, 1975a, 1975b, 1977; Worth et al. 1975), Brunei and Sabah (Maclean 1979) and, recently in Palau (Harada et al. 1982).

At the height of and even a month before the bloom, several cases of paralytic shellfish poisoning (PSP) in humans were reported mainly due to the ingestion of the green mussel, *Perna viridis* L., the black-lipped pearl oyster, *Pinctada margaritifera*, the squid, *Loligo* sp., and, at times, the giant clam, *Tridacna* sp. (Estudillo 1983; Estudillo and Gonzales 1984). Although the official tally stood at 21 deaths and 278 cases of hospitalization (Estudillo and Gonzales 1984), the number of fatalities and milder cases might have been more; reports were not coordinated. A newspaper report claimed a total of 49 deaths and a large number of mild intoxications (Ensoy 1983).

There is no known completely effective antidote to *P. bahamense* var. *compressa*-caused PSP. However, many victims were relieved of the symptoms by drinking pure coconut milk (*gata* in Pilipino) with brown sugar, or sugar lumps (*tagapulot* in Pilipino) (Ensoy 1983; Estudillo 1983). This study determines the extent of detoxification in crude extracts of PSP toxin from *P. viridis* when reacted *in vitro* with either coconut milk or brown sugar or both.

Materials and Methods

The extraction of crude toxin was patterned after the AOAC method (Association of Official Agricultural Chemists 1965).

The treatments tested in the experiments were (a) crude toxin (CT), (b) coconut milk (CM), (c) crude toxin + coconut milk (CT + CM), (d) crude toxin + coconut milk + brown sugar (CT + CM + BS), (e) crude toxin + brown sugar (CT + BS), and (f) brown sugar (BS). The diluent in (b) and (f) was 0.1 N hydrochloric acid. In all cases, 5 ml of the crude toxin and of the acid were prepared.

Prior to any dilution, the crude toxins were homogenized thoroughly. Several 5-ml volumes of each crude toxin were placed in four glass vials (for a, b, d and e).

Into each vial intended to contain CM, 0.25 g of the powdered, desiccated coconut milk was added and mixed thoroughly. A similar weight was used for the brown sugar. The concentration of both CM and BS was 5% (w/v) in all cases. The mixtures were reacted under room temperature (28°C) for one hour.

With hydrochloric acid, the pH in each vial was adjusted to 3.0 just before the injections. It was at this pH value that extracts were most potent (Arafiles et al. 1984). Quantifications of the resultant toxin were made using the standard mouse bioassay (AOAC 1965). Whenever possible, three mice were injected for each treatment.

Results

The results of the bioassays are presented in Table 1. All indications point to a reduction in the toxicity of the crude toxins after a one-hour reaction with either CM or BS, or CM + BS. The crude toxin with an initial mean toxicity of 2,114 MU/100 g meat (CT-a), when reacted with CT + CM, had a resultant mean toxicity of 664 MU/100 g. The reduction in toxicity was 68.5%. The resulting toxicity in CT + CM + BS was 1,005 MU/100 g meat. The reduction in toxicity in this case was 52%.

CT-b, with a mean toxicity of 498 MU/100 g meat (436-536 MU/100 g) was also detoxified judging from the results. One mouse died within 23 min. and 31 sec. in CT + BS. Except for this, no other deaths were observed within one hour in the other treatments. CT (CT-c) with a toxicity of 183 MU/100 g (128-231 MU/100 g) also produced no deaths within one hour when reacted with either CM, BS, or CM + BS.

Discussion

The toxicity of molluscs previously exposed to blooms of *Pyrodinium bahamense* var. *compressa* in Southeast Asian and western tropical Pacific countries is often high. This could be glimpsed from results with the standard bioassay. In Papua New Guinea, peak mouse death times were 1 min., 30 sec. with *Barbatia paravillosa* (Maclean 1975b) and 1 min., 18 sec. with *Spondylus* sp. (Maclean 1975b). These times should have placed the toxicities at 3,130-3,470 MU/100 g and 5,280 MU/100 g meat, respectively. The highest recorded toxicity in Brunei was 2,310 MU/100 g involving a lamellibranch (Jaafar and Subramanian 1984); in Sabah, it was 9,920 MU/100 g for a giant clam (Wong and Ming 1984). In the Philippines, the highest toxicity (9,620 MU/100 g) was obtained with *Perna viridis* collected in October 1983 from Balete Bay, Mati, Davao Oriental (Gacutan et al. 1985). All these values, however, would pale in comparison with the toxicity of *Spondylus butleri* (11,100 MU/100 g) collected from Arumizu Bay, Palau in May 1980 (Harada et al. 1982).

In this experiment, the toxicity of crude toxin extracts was sufficiently reduced after an hour's reaction with either coconut milk or brown sugar or both. The reduction in a crude extract with toxicity of 2,114 MU/100 g meat amounted to 68.5% when powdered desiccated coconut milk was added to the toxin at 5% (w/v); and 52% when brown sugar was added also at 5% (w/v). One could safely say then, that the human fatalities would have been more in the aftermath of the bloom in Western Samar and Leyte had the palliative properties of these products against PSP not been known.

Filipinos prefer to eat molluscs raw, slightly cooked or blanched. A check with medical authorities in Western Samar and Leyte, as well as interviews with the populace, showed that a good number of the fatalities and of those hospitalized ate mussels and fish cooked in vinegar or mussels simply broiled or steamed and dipped in vinegar. The PSP victims also recovered faster with coconut milk and brown sugar when the mussels or fish were prepared and eaten without the use of vinegar.

The phenomenon of potentiation similar to what was demonstrated by researchers (Harada et al. 1984) may have worked in the above cases. It was shown that one of the toxins present, normally a low toxicity component, was converted into a potent form in a dilute acid environment, increasing in toxicity by as much as 15-fold (Harada et al. 1982b). Other studies showed lower potentiation of toxicity (Nishio et al. 1982).

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Table 1. Results of the bioassays using three crude toxin extracts. The mean toxicities of the extracts were as follows: CT-a, 2,114; CT-b, 498 and CT-c, 183 MU/100 g meat. When the mouse did not die within one hour, the result was reported as "dnd" (did not die).

	CT-a Replicates			CT-b Replicates				CT-c Replicates		
	1	2	3	1	2	3	4	1	2	3
CT	2,043	1,762	2,581	544	251	563	436	128	192	231
CM	dnd*	dnd	dnd	dnd	dnd	dnd		dnd	dnd	dnd
CT+CM	481	847		dnd	dnd	dnd	–	dnd	dnd	dnd
CT+CM+BS	924	882	1,198	dnd	dnd	dnd	–	dnd	dnd	dnd
CT+BS	784	568	595	91**	dnd	dnd	–	dnd	dnd	dnd
BS	dnd	dnd	dnd	dnd	dnd	dnd	–	dnd	dnd	dnd

*Did not die within one hour after injection.

**One mouse died after 23 min., 31 sec.

Trace Metals in Pacific Oysters (*Crassostrea gigas* Thunberg) Marketed in Hong Kong

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Abstract

A two-year study of trace metal concentrations in oysters (*Crassostrea gigas* Thunberg) marketed at Lau Fau Shan in Hong Kong is reported. Comparison of the data with previous studies reveals a decrease in the average concentrations of cadmium, copper and mercury in marketed oysters in recent years. However, the concentrations of lead and zinc have not altered substantially during this period. The long-term changes noted may be related to restocking of local oyster beds subsequent to a mass mortality in 1979, and/or to altered importation practices. With the exception of cadmium, no sample studied contained element concentrations exceeding the maximum permitted level under present Hong Kong legislation. However, the maximum permissible concentration for cadmium in foods (2.0μg/g wet weight in the case of oysters) was exceeded by 18.8% of the samples analyzed. The possible significance of this with respect to the protection of public health is discussed.

Introduction

The Pacific oyster (*Crassostrea gigas* Thunberg) is cultured commercially in Hong Kong and China. Information on the Hong Kong oyster industry is available in a variety of published reports, dealing with the culture itself (Bromhall 1958; Mok 1973, 1974a, 1974b; Morton and Wong 1978), biological and ecological aspects (Ko et al. 1975; Wong 1975; Morton 1977), fecal contamination of the beds (Morton 1975; Leung et al. 1975; Morton and Shortridge 1976), and trace elements in the oysters (Wong et al. 1981; Phillips et al. 1982).

The concentrations of trace metals in *C. gigas* cultured or marketed in Hong Kong are of importance, as previous studies have indicated that potential problems exist with respect to public health. In particular, the cadmium concentrations found in oysters marketed in Hong Kong during 1978 commonly exceeded the present local legislative limit of 2.0μg/g wet weight laid down in the Food Adulteration (Metallic Contamination) Regulations under the Public Health and Urban Services Ordinance. By contrast, *C. gigas* samples taken directly from the Deep Bay beds in mid-1978 contained lower levels of cadmium which were within currently permissible limits. This difference was ascribed to the effects of the importation of oysters more heavily contaminated by cadmium than the local product (Phillips et al. 1982).

In 1979, oysters grown in the Hong Kong Deep Bay beds suffered a mass mortality estimated as 90% or more. The importation of oysters from China accounted for almost all of *C. gigas* marketed in Hong Kong in late 1979 and early 1980. However, the local farmers restocked the beds in Deep Bay in 1980 with imported oysters (of a variety of sizes/ages) and local production resumed. The present studies were initiated to investigate the influence of these events on the trace element concentrations in *C. gigas* marketed in Hong Kong, and in particular to elucidate any long-term changes which may have occurred since 1978.

Materials and Methods

Samples of oysters (*C. gigas* Thunberg), 9-33 specimens depending on size, were purchased monthly at Lau Fau Shan retail market (northwest New Territories) from December 1981 for two years. The samples were representative of the oysters available at the market when visited, as on all occasions, one sample was purchased from each stall offering oysters. All samples purchased were shucked at the market by the stallholder, to reflect the normal conditions of purchase by the public. In the laboratory, wet tissue weights of the whole soft parts of each individual were recorded, and four subsamples (each containing several oysters) were taken. The subsamples were stored at -20°C.

Upon thawing, each subsample was thoroughly homogenized using a commercial blender (shown in previous studies to be non-contaminating). Aliquots of the homogenate were taken for the analysis of cadmium, copper, chromium, lead, mercury and zinc. These aliquots were digested with nitric or nitric and sulphuric acids and suitably diluted thereafter. Samples were analyzed by atomic absorption spectrophotometry, using a Hitachi 180-80 Zeeman instrument in flame mode or a Perkin Elmer PE 4000 with graphite furnace. Mercury was analyzed on a Perkin Elmer PE 50A analyzer, using the cold vapor generation technique. Quality control was assured by the concurrent analysis of various reference and intercomparison materials; in particular, the albacore tuna RM 50 material supplied by the National Bureau of Standards (Washington, USA) was commonly employed during these studies. All final concentrations of elements are based upon wet tissue weights, as are the present Hong Kong legislative limits for trace elements in foodstuffs.

Results

Concentration-frequency plots for all elements other than mercury in the oysters sampled are shown in Figs. 1-5 for the two years (December 1981 to November 1982 and December 1982 to November 1983) of the survey, in addition to the overall profile. For mercury, 86.7% of the samples were found to contain concentrations close to or below the detection limit of $0.02\mu g/g$ wet weight. The highest mercury concentration recorded was $0.07\mu g/g$ wet weight, in one sample purchased in October 1982.

Concentrations of cadmium found in the oysters sampled ranged from 0.23 to $5.88\mu g/g$ wet weight. There was little difference between results from the two years. The percentage of samples exceeding $2.0\mu g/g$ wet weight (the present legislative limit in Hong Kong) was 18.8%. Concentrations of chromium varied between 0.02 and 0.43 $\mu g/g$ wet weight in sampled oysters. There was a tendency towards higher concentrations of this element in the first year of study, although this was not marked. Copper concentrations ranged from 12 to $495\mu g/g$ wet weight. Concentration-frequency plots for copper were similar for the two years, with most samples containing $30\text{-}150\mu g/g$. Levels of lead found in samples ranged from 0.01 to 1.0 $\mu g/g$ wet weight; again, oysters from the first year of study tended to exhibit higher concentrations. Finally, zinc concentrations varied between 106 and $1{,}360\mu g/g$ wet weight, and concentration-frequency plots were similar in each year.

Discussion

These studies demonstrate the great variability in trace element levels found in *C. gigas* marketed in Hong Kong. The concentrations of all metals other than mercury in this study varied by greater than an order of magnitude in overall terms. This variability is a common feature of metals in molluscs, and in particular bivalves (e.g., see Bryan 1976; Phillips 1977, 1980), and is caused by the general lack of metabolic regulation of trace elements amongst these species. Thus, the metal levels found in bivalves commonly vary not only with season and various other factors (e.g., size, position on shore line, sex), but also with location, in response to the variable ambient concentrations of elements in water and sediments. This has given rise to the use of bivalves as so-called biological indicators or "sentinel" organisms, to provide a time-integrated picture of the degree of contamination of coastal waters by trace elements and other marine pollutants (Phillips 1980).

The main causes of variability in the trace element concentrations present in oysters marketed at Lau Fau Shan are likely to be seasonal changes in metal levels and the influence of the location of culture. However, the effects of these two factors cannot be fully separated from each other in the present study. This is because data from retailers of oysters concerning the area of culture of their product are most unreliable.

Phillips et al. (1982) reported that oysters sampled in mid-1978 from Deep Bay exhibited different concentrations of certain elements, particularly cadmium, from those in oysters purchased at Lau Fau Shan market in the same period. Samples from Deep Bay in 1978 exhibited cadmium levels of 0.42 to $1.12\mu g/g$ wet weight (mean ± S.D. = $0.77 \pm 0.18\mu g/g$), whereas oysters from Lau Fau Shan market contained 0.6 to $5.4\mu g/g$ wet weight (mean ± S.D. = $2.79 \pm 1.24\mu g/g$). This difference was ascribed to the importation of oysters more heavily contaminated by cadmium than the local product, and the dominance of the cadmium-enriched oysters in retail markets during the summer (when local oysters are rarely harvested; see Phillips et al. 1982). Results from the present studies show that the mean cadmium concentration in oysters marketed at Lau Fau Shan has decreased since 1978; in 1981/82 the overall mean was $1.48\mu g/g$ wet weight and in 1982/83, $1.45\mu g/g$ wet weight.

Significant decreases have also occurred since 1978 with respect to the concentrations of copper and mercury in marketed oysters at Lau Fau Shan. In 1978, marketed oysters averaged $194\mu g/g$ wet weight for copper, in 1981/82, $97\mu g/g$ wet weight, and in 1982/83, $85\mu g/g$ wet weight. For mercury, oysters from the market in 1978 averaged $0.21\mu g/g$ wet weight with a maximum of $1.26\mu g/g$

in one sample; however, no sample in the present study exceeded 0.07μg/g wet weight.

In contrast, the levels of lead and zinc remained similar from 1978 to 1983. Mean lead concentrations recorded in oysters purchased in 1978 were 0.22μg/g wet weight, compared to 0.34μg/g in 1981/82 and 0.24μg/g in 1982/83. For zinc, oysters taken in 1978 exhibited mean concentrations of 419μg/g wet weight, in 1981/82, 338μg/g and in 1982/83, 380 μg/g. No data for the levels of chromium in oysters were reported in the 1978 studies.

The acquisition of accurate data on oyster marketing practices is extremely difficult, partly due to the complexity of the industry. It is believed that the oyster tonnage imported for direct marketing through Lau Fau Shan (as opposed to imports for transplantation into Deep Bay) has diminished considerably in recent years (unpublished data from Agriculture and Fisheries Department, Hong Kong Government). It is possible that greater emphasis has been accorded to marketing the locally grown (Deep Bay) product since the restocking of the Bay subsequent to the 1979 mass mortality. This hypothesis is supported by unpublished data on cadmium concentrations in oysters taken directly from Deep Bay in January and February 1984 which exhibited relatively low cadmium concentrations of 0.5 to 1.6μg/g wet weight, agreeing closely with 1978 data on locally grown *C. gigas* (Phillips et al. 1982). Oysters from Sha Tsing in the Pearl River Estuary at that time contained cadmium levels of 2.3 to 2.6μg/g wet weight.

The Food Adulteration (Metallic Contamination) Regulations under the Public Health and Urban Services Ordinance lay down maximum permissible concentrations for trace elements in foods marketed in Hong Kong, including marine products. The recent amendment to these Regulations (enacted in 1983) included limits for antimony, arsenic, cadmium, chromium, lead, mercury and tin. No maxima were set for copper and zinc levels in foods, as these are of relatively minor public health hazard although zinc levels considerably in excess of those reported here caused an emetic effect when Tasmanian oysters were ingested in any number (Ratkowsky et al. 1974). None of the elements in marketed oysters exceeds the present legislative maximum concentrations for foods, with the exception of cadmium. Some 18.8% of the samples of *C. gigas* analyzed in this study contained cadmium concentrations which exceeded the limit of 2.0 μg/g wet weight. The highest concentration recorded throughout the study was 5.88μg/g wet weight, almost three times the allowable maximum. Although these data compare favorably with the situation in 1978, there remains possible cause for concern with respect to the potential effects on public health. Although cadmium is known to be unusually persistent in humans (biological half-life of 16 to 33 years; Friberg et al. 1974) and provisional allowable weekly intakes of the element have been proposed at 400-500μg (WHO 1972), epidemiological evidence from populations exceeding this exposure rate reveal no clear picture of toxic hazard (CEC 1978).

The data reported here show that some 18.8% of locally retailed oysters exceeded the present legislative limit for cadmium of 2.0μg/g wet weight. The arithmetic and geometric means for the data set are 1.49 and 1.35μg/g wet weight, respectively, both significantly lower than the present public health limits. An Australian committee concerned with mercury in seafoods (AFC 1979) posited that public health limits could be treated in at least some cases as means across catches, rather than as maxima for individuals. It should be noted, however, that this approach involves assumptions not only on the safety margins which are acceptable for public health limits, but also on the randomization of the highest contaminated items throughout the retail market. In addition, this approach is only a realistic option when sufficient baseline data are available on contaminant incidence in seafoods and when the major sources of variation are both known and controllable. It would be exceedingly difficult to administer the cadmium regulations with respect to Hong Kong oysters in this fashion, particularly in view of the problems of obtaining accurate information on oyster marketing procedures.

The long-term changes reported here for trace element concentrations in *C. gigas* marketed in Hong Kong are significant in terms of public health. There is an argument for further monitoring, particularly because it is possible that the proportion of imported oysters may alter again in the future, with consequent changes in trace metal levels and because the trace element concentrations present in *C. gigas* from the different beds vary quite markedly.

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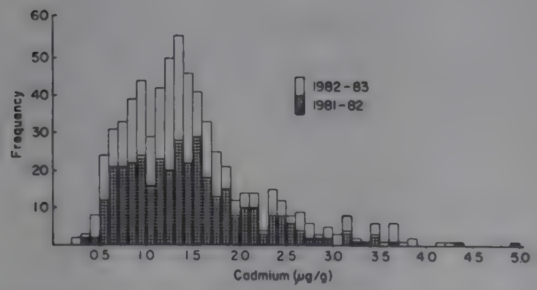

Fig. 1. Concentration-frequency plot for cadmium in whole soft parts of oysters *(Crassostrea gigas)* purchased from Lau Fau Shan market between December 1981 and November 1983. One sample containing 5.88 µg/g not shown.

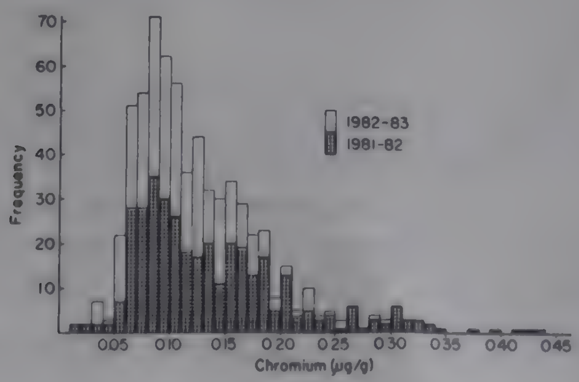

Fig. 2. Concentration-frequency plot for chromium in whole soft parts of oysters *(Crassostrea gigas)* purchased from Lau Fau Shan market between December 1981 and November 1983.

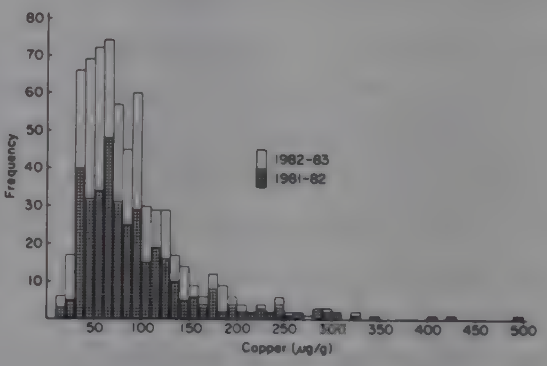

Fig. 3. Concentration-frequency plot for copper in whole soft parts of oysters *(Crassostrea gigas)* purchased from Lau Fau Shan market between December 1981 and November 1983.

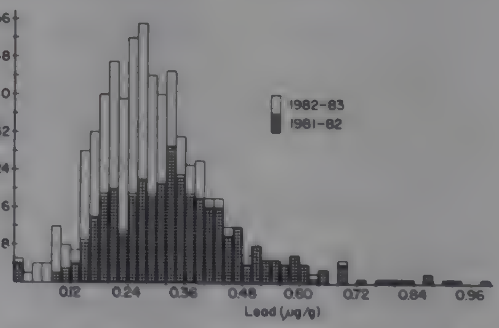

Fig. 4. Concentration-frequency plot for lead in whole soft parts of oysters *(Crassostrea gigas)* purchased from Lau Fau Shan market between December 1981 and November 1983.

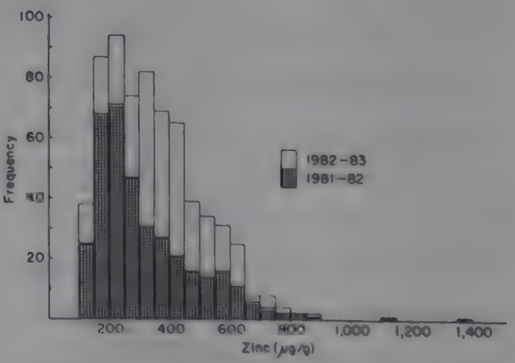

Fig. 5. Concentration-frequency plot for zinc in whole soft parts of oysters *(Crassostrea gigas)* purchased from Lau Fau Shan market between December 1981 and November 1983.

Toxicity of Metal Mixtures to *Perna viridis* (Mollusca: Pelecypoda)

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Abstract

Perna viridis when exposed to a mixture of zinc and copper recorded enhanced toxicity of one metal in the presence of another metal. This reflects the increased permeability of the plasma membrane in the presence of toxicant combinations which, in turn, brings about elevation in toxic response. When the experimental medium contained 0.02 ppm copper and 2.37 ppm zinc, the test individuals recorded 50% mortality in 96 hours. Elevation in copper concentration to 0.08 ppm in conjunction with 1.25 ppm of zinc caused 50% mortality in 96 hours.

Introduction

Available reports on the toxicity of heavy metals on marine invertebrates usually try to explain the toxicity of a single metal pollutant (Connor 1972; Calabrese et al. 1973). In nature, pollutant emissions seldom, if ever, consist of a single toxicant. Such emissions usually contain a mixture of metals and, consequently, result in the exposure of the indigenous biota to several metals. The response of the biota to individual metals may differ from the response to stress from multiple metals as indicated by several studies (Mac Innes 1981; Murthy 1982; Mathew and Menon 1983; Prabhudeva 1983; Mohan and Menon, in press). The studies have evaluated the potential antagonistic, synergistic or additive interactions of metal mixtures. Antagonistic interactions refer to the protective effect of one metal on the toxicity of another and are probably a reflection of the competition between the two metals for sites of entry in the cell surfaces, with a reduction in the subsequent uptake and accumulation of both metals. Synergistic interactions refer to the enhanced toxicity of one metal in the presence of another and may reflect the increased permeability of the plasma membrane when stressed by several toxicants. Additive interactions are neither antagonistic nor synergistic, and the final toxicity is simply a sum of the individual toxicities.

Materials and Methods

Green mussels, *Perna viridis* were collected from rocky shores of Someshwara, South India. They were conditioned in the laboratory (water temperature, 31 ± 1°C; salinity 32 ±0.5 ppt; pH 8.2-8.4) for a period of 48 hours, before being employed for the experiments. Mussels of 15-20 mm shell length were used.

Individually prepared zinc ($ZnCl_2.2H_20$) and copper ($CuSo_4.5H_20$) solutions formed the source of metals. The experimental vessels were cylindrical glass troughs of 5-liter capacity containing 4-l test solution prepared in aged seawater. Mortality tests were performed over 96 hours. The inability to close the valves upon mechanical stimulation and/or valve gaping of 5 mm were the criteria used to define the death of the test organisms. Dead individuals were removed from the experimental media at 12-hour intervals. Ten mussels were exposed to each metal combination.

The cumulative percentage mortality of *P. viridis* exposed to combinations of zinc and copper was estimated in two sets of experiments in duplicate, one with zinc concentrations ranging from 1.5 to 3.5 ppm in combination with a constant copper concentration of 0.02 ppm and another where the same concentration of zinc was maintained along with 0.08 ppm of copper. All the concentrations reported here are the calculated levels of metal ion added at the start of the experiment and do not include natural levels. Actual concentrations of each metal in the test cultures were not determined.

The experimental vessels were not aerated, but the oxygen level in the water was monitored. The pH levels after adding the metal salts in all vessels ranged from 8.2 to 8.4 throughout the study. The lines of best fit were drawn after linear regression analysis. An additive toxicity index developed by Marking and Dawson (1975) was used to determine the toxicity of metal mixtures.

Results

The maximum mortality recorded was 90% in experimental media where the zinc concentration was 3.0

and 3.5 ppm with 0.02 ppm of copper. The 96-hour LC_{50} was 2.37 ppm of zinc (95% confidence limits: 2.056-2.76 ppm) with 0.02 ppm of copper (Fig. 1).

In the next series of experiments, the same concentration of zinc was maintained and the copper concentration was increased to 0.08 ppm. Total mortality occurred in 96 hours at 2.5 and 3.5 ppm of zinc. This brought down the 96-hour LC_{50} to 1.25 ppm of zinc (95% confidence limits: 1.087-1.43 ppm) with 0.08 ppm of copper (Fig. 2).

The joint action of the zinc plus copper mixture on mortality of *P. viridis* was worked out, and the results are both more than additive and less than additive in nature. It was more than additive (+0.42 additive index) with low concentration of copper (0.02 ppm) and less than additive (-0.033 additive index) with high concentration (0.08 ppm) in combination with the same concentration of zinc.

Discussion

The toxicity of chemical combinations is usually assessed by adding the concerned chemicals in a definite ratio which is decided by the chemicals' individual toxicity. Although such a procedure is possible under controlled laboratory conditions, it is quite unlikely that such a situation exists in nature. Therefore, information from literature usually pertains to toxic mixtures where one of the chemicals will be retained at a definite constant concentration and the other treated as a variable. Similarly, in the present investigation two sets of experiments were performed where copper was administered to the medium at 0.02 and 0.08 ppm with different zinc concentrations.

Discussing the combined toxicity of silver and copper on *P. viridis*, Mathew and Menon (1983) stated that the metal concentration of 0.031 ppm of copper and 0.031 ppm of silver brought about 50% mortality of this species in 96 hours. They also found that silver in combination with copper becomes more toxic. Similarly, Mac Innes and Calabrese (1978) in a detailed study on the effect of mercury and silver, and zinc and copper individually and in combination, on the embryos of *Crassostrea virginica* found that these two mixtures exerted a less than additive toxicity at low temperatures. Discussing the combined cadmium and zinc toxicity on embryonic development of *Mytilus galloprovincialis*, Pavicic (1977) opined that a combination of cadmium and zinc increased the toxic resistance of the embryos.

The individual 96-hour LC_{50} for *P. viridis* was 4.74 ppm (95% confidence limits: 5.36-4.20) of zinc and copper 0.105 ppm (95% confidence limits: 0.104-0.106 ppm). Here, the presence of 0.02 ppm of copper in the toxicity test medium enhanced the toxicity of zinc twofold, while the presence of 0.08 ppm of copper increased the toxicity of zinc 3.79 times. It is common to find the toxicity of a mixture of metals synergistical, antagonistical or simple additive. These reactions could be species specific, or could be influenced by environmental alterations. It is evident that zinc, in combination with copper, becomes more toxic. Mac Innes (1981) noticed either antagonistic effect or simple additivity in lowest metal concentrations of copper + mercury, copper + zinc, copper + mercury + zinc, and mercury + zinc. But as the metal concentrations increased, the effect becomes synergistic, and this was so for copper and zinc. Mac Innes (1981) felt that this may be due to the complexing capacity of seawater, resulting in a low availability of heavy metals. Similarly, our results showed more than additivity (+0.42 additive index) at low concentration of copper (0.02 ppm) and less than additivity (-0.033 additive index) at high concentration (0.08 ppm of copper) in combination with the same concentrations of zinc. Mohan and Menon (in press) found a more than additive interaction between mercury and cadmium on *P. viridis*. Moulder (1980) proved the antagonistic nature of copper on mercury toxicity using synthetic seawater devoid of organic ligands. The alterations in toxicity recorded here could be due to the capacity of *P. viridis* to selectively block the binding site of such metals to which they have high resistance as suggested by Moulder (1980) and Breittmayer and Gutierrez-Galindo (1981).

Acknowledgements

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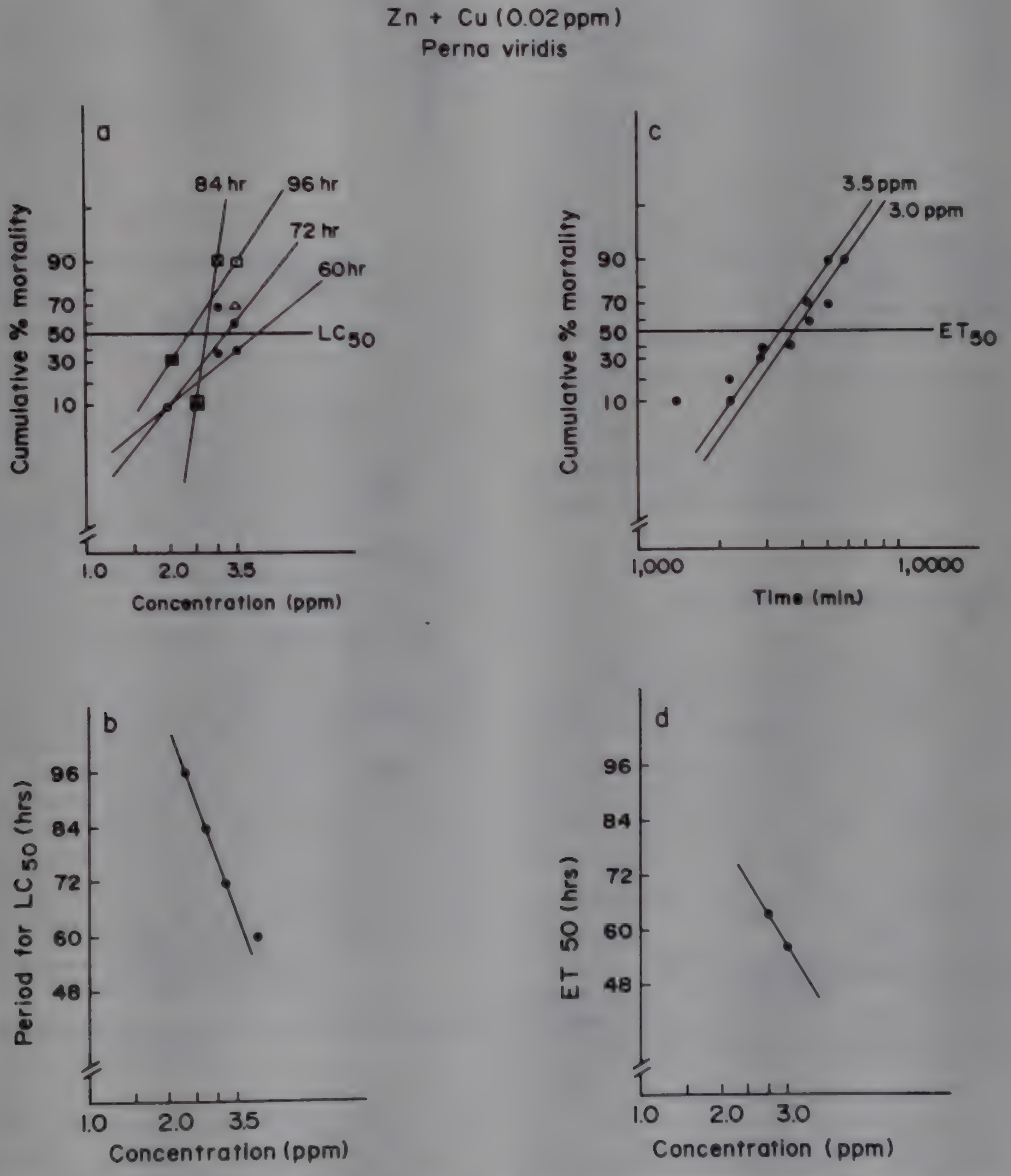

Fig. 1. Lethal effects at 0.02 ppm of copper along with different concentrations of zinc. a. Progress of mortality against concentration. b. Progress of mortality against time. c and d. Toxicity curves.

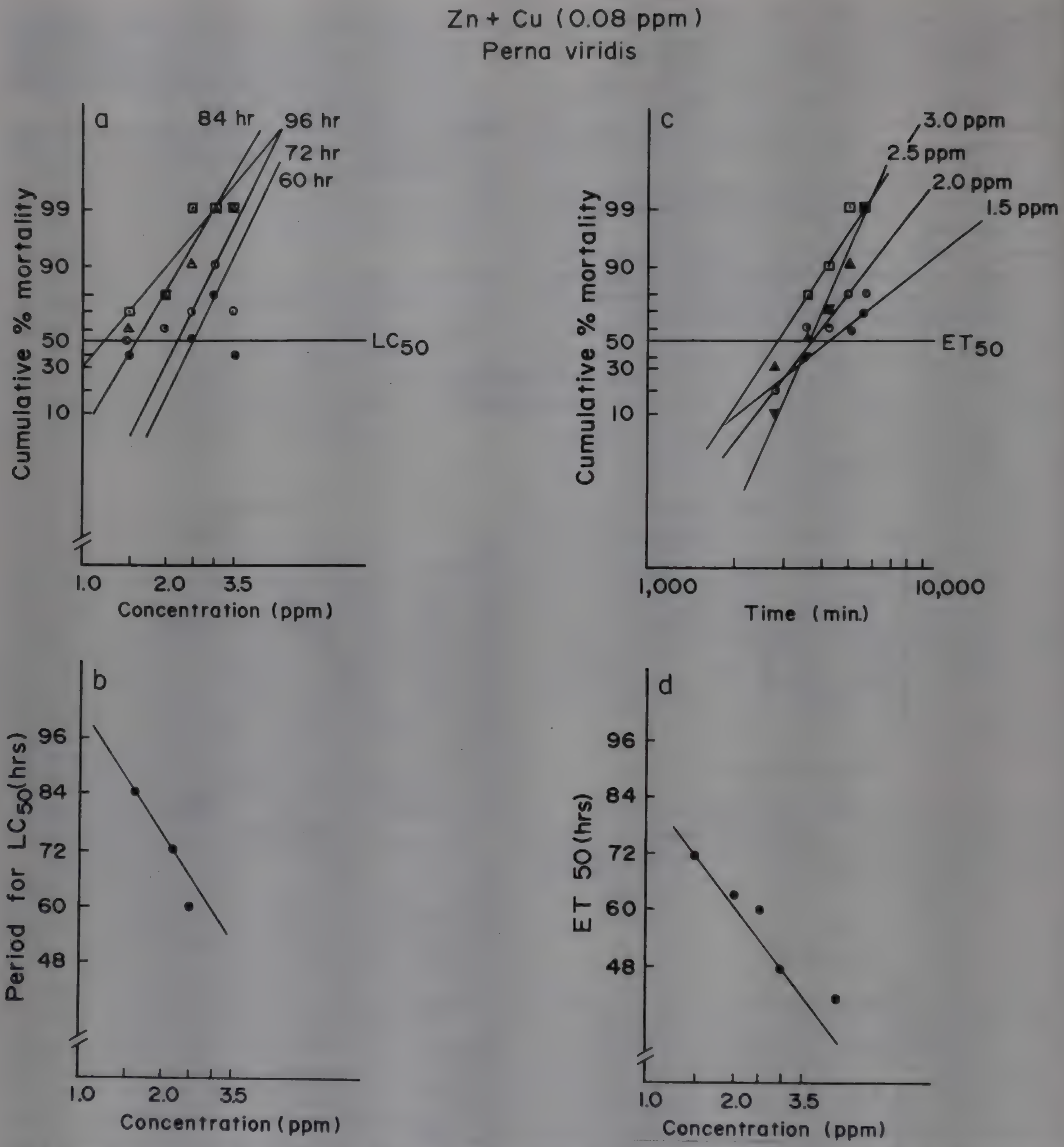

Fig. 2. Lethal effects at 0.08 ppm of copper along with different concentrations of zinc. a. Progress of mortality against concentration. b. Progress of mortality against time. c and d. Toxicity curves.

Isolation of a New Rhabdovirus from Cultured Hirame (Japanese Flounder, *Paralichthys olivaceus*) in Japan

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Abstract

In March 1984, a new rhabdovirus was isolated from moribund cultured hirame (Japanese flounder, *Paralichthys olivaceus*) and from ayu *(Plecoglossus altivelis)* fry in Hyogo Prefecture. From February through May 1985, the virus was again isolated from hirame in seawater tanks in Hyogo and Kagawa Prefecture, and in Hokkaido, Japan. At temperatures between 5 and 20°C the virus replicated and induced cytopathic effects (CPE), which progressed to eventual cytolysis in susceptible cell lines, including FHM, EPC, BF-2, RTG-2, STE-137, HF-1, BB, YNK, CCO and EK-1. The CHSE-214, KO-6 and CHH-1 cell lines were refractory. Maximum infectivity of cell culture-grown virus was about $10^{9.3}$-$10^{9.8}$ $TCID_{50}$/ml in FHM or EPC cell lines. The virus replicated optimally at 15 to 20°C. Virus particles were bullet shaped, 80 nm x 180 to 200 nm. The isolate was sensitive to pH 3, to diethyl ether, and to heat (50°C, 2 min.). The virus did not hemagglutinate human O type erythrocytes. Viral replication was not inhibited by 10^{-4} M 5-iododeoxyuridine. Infectivity was not reduced by antisera against infectious hematopoietic necrosis virus (INHV), viral hemorrhagic septicemia virus (VHSV), spring viremia of carp virus (SVCV), pike fry rhabdovirus (PFRV), eel virus-American (EVA) and eel virus-European (EVX). The viral isolate was pathogenic for hirame and rainbow trout *(Salmo gairdneri)* by injection but not for chum salmon *(Oncorhynchus keta)*, coho salmon *(O. kisutch)*, masu salmon *(O. masou)*, or ayu fry by water borne exposure. From the evidence obtained thus far, this virus is a new pathogenic virus of fish, and is provisionally named HRV (hirame rhabdovirus). HRV is now considered to be an important pathogen of cultured hirame and salmonids in Japan.

Introduction

An unknown disease occurred during March 1984 among pen-cultured hirame *(Japanese flounder, Paralichthys olivaceus)* held in pens in Fukura Bay, and among cultured ayu *(Plecoglossus altivelis)* fry held in seawater tanks at the Hyogo Prefectural Fisheries Experimental Station, Hyogo Prefecture (Gorie et al. 1985a, 1985b). The signs of disease in hirame were congestion of the gonads, focal hemorrhage in the skeletal muscles and fins, and accumulation of ascitic fluid. Infected ayu fry exhibited exophthalmia and pethechia on the gillcover. The following year, February to May 1985, the same disease occurred again among hirame cultured in seawater tanks at the Hyogo Prefectural Fisheries Experimental Station, Negi Island, Kagawa Prefecture and Yagishiri Island, Hokkaido. Examination of diseased fish failed to show any bacterial, fungal or parasitic agents.

However, during the course of viral examination, infectious hematopoietic necrosis virus (IHNV)-like cytopathic effect (CPE) was observed in the RTG-2 cell cultures. This agent was not neutralized with antisera against the following fish rhabdoviruses: IHNV, viral hemorrhagic septicemia virus (VHSV), spring viremia of carp virus (SVCV), pike fry rhabdovirus (PFRV), eel virus-American (EVA) and eel virus-European (EVX). Pathogenicity and virulence of the agent to the hirame was demonstrated by intraperitoneal injection (Gorie et al. 1985a).

This report describes the characteristics and pathogenicity of the new virus provisionally termed hirame rhabdovirus (HRV).

Biophysical Characterization

Biophysical characteristics of the HRV 8401H originating from diseased hirame and HRV A-8401H isolated from moribund ayu fry are summarized in Table 1. Ether treatment markedly reduced infectivity of the viruses. The BSS treated control had a titer of $10^{6.05}$ TCID 50/ml while the ether-treated suspension had less than $10^{1.80}$ TCID 50/ml. This sensitivity to ether is evidence that the virus has an essential lipid-containing envelope.

The deoxyuridine analogue IUdR was not effective in blocking viral replication. Control cultures and IUdR treated cultures had similar titers ($10^{6.05}$ $TCID_{50}$/ml). The lack of inhibition by halogenated pyrimidines is evidence that the virus possesses an RNA genome.

Both isolates were inactivated by exposure to pH 3. No hemagglutination by HRV was observed with human O type erythrocytes.

Electron Microscopy

Virus particles were seen in ultrathin sections of HRV infected RTG-2 cells and the kidney tissue of HRV infected hirame. The particles were bullet shaped with one rounded end and a mean diameter of 80 nm and length of 180-200 nm. These morphological and biophysical features are characteristic of viruses of the Rhabdoviridae.

Serological Characterization

The five strains of HRV used were isolated from two host species at three different locations over a two-year period. All were neutralized with anti-HRV 8401H rabbit serum. No neutralization of HRV infectivity by antisera against IHNV, infectious pancreatic necrosis virus (IPNV), *Oncorhynchus masou* virus (OMV), VHSV, SVCV, PFRV, EVA or EVX was observed. The infectivity of each control virus used was reduced over 100-fold by the homologous antiserum.

Cross neutralization tests indicated that HRV was clearly distinguishable from the five reference rhabdoviruses (Table 2). HRV was neutralized with homologous antiserum at a titer of 160 and not neutralized with the heterologous antisera tested. Anti-IHNV, SVCV, EVA and EVX rabbit sera also had the highest titer against homologous virus. Cross reactions were not recognized except between EVA and EVX.

Cell Line Susceptibility and Virus Replication

The HRV was originally isolated using RTG-2 cells. Twelve other fish cell lines (FHM, EPC, BF-2, STE-137, HF-1, BB, YNK, CCO, EK-1, CHH-1, CHSE-214 and KO-6) were also tested for HRV susceptibility. The EPC, FHM, BF-2, YNK, STE-137, BB, EK-1, CCO and HF-1 cell lines were susceptible to HRV infection. The EPC and FHM cell lines showed the greatest sensitivity, while no CPE developed in CHH-1, CHSE-214 and KO-6 cells (Table 3). These latter three cell lines were susceptible to IHNV, but the HF-1, BB, EK-1 and CCO cells were not.

As judged by virus yield (Fig. 1), the FHM and RTG-2 cells were the most efficient at replicating HRV. An incubation time of 72-120 hours was required to observe CPE in EPC, HF-1, STE-137, BF-2 cells and the virus yields were greater than $10^{8.80}$ TCID $_{50}$/ml. No CPE developed in CHSE-214, CHH-1 or KO-6 cells after 10 days incubation at 15°C.

The temperature range for viral replication was determined to be 5 to 25°C. The RTG-2 cell line was used for those experiments performed at 5 to 20°C while the EPC cell line was used at 15 to 25°C because it tolerates a higher temperature. The extent of CPE correlated with the virus titer produced over the range of 5 to 20°C. At 10°C, viral replication was slower and CPE less extensive than at 15 or 20°C. At 10°C, viral replication was slower and CPE less extensive than at 15 or 20°C. In the case of IHNV and IPNV, the optimal temperatures were 15°C and in the range of 15 to 20°C, respectively.

Pathogenicity of HRV

The pathogenicity of the virus was tested for specific pathogen-free chum salmon *(Oncorhynchus keta)*, masu salmon *(O. masou)*, coho salmon *(O. kisutch)*, rainbow trout *(Salmo gairdneri)* and ayu. Ten rainbow trout (mean weight approximately 8 g), and masu salmon (6 g) were injected intramuscularly with $10^{4.0}$ TCID$_{50}$/fish of virus, and fish were held in 20-l aquaria at 12-13°C for 21 days. Fifty chum and coho salmon, thirty masu salmon, and fifteen ayu, weighing approximately 0.2, 0.5, 0.2 and 0.5 g, respectively, were infected by the immersion method using $10^{3.0}$ TCID$_{50}$/ml. Fish were exposed to the virus at 10°C for 60 min and then held in 3-l aquaria at 12-14°C. Ayu were maintained at 16-17°C for 21 days. After 21 days, five fish, including one that died, were removed and examined for bacteria and virus.

Mortality among rainbow trout began at six days post-injection and 60% had succumbed by day 12. Hemorrhages were commonly observed in muscle and fin. The cumulative mortality of control fish was 10% in 21 days. For masu salmon 1 of 10 control fish and 0 of 10 virus injected fish died during the experiment. In coho salmon fry, no control or experimental fish died. Among chum salmon, 3 of 50 control fish and 5 of 50 virus exposed fish died. No control and 2 of 30 virus immersed masu salmon fry died during the 21-day period. Among the ayu no control or virus-exposed fish died in the experiment (Table 4).

Virus isolation was carried out using five fish from each group, composed of mortalities or fish sacrified at the end of the experiment. Virus was recovered from five individual rainbow trout (all mortality), one masu salmon fry (mortality), one masu salmon fry (sacrificed and three coho salmon fry (sacrificed). Signs of disease were only observed in rainbow trout. All the isolates were neutralized with anti-HRV serum.

Virus titers in tissues of infected rainbow trout were determined for kidney and spleen pools, liver and pancreas pools, heart, intestine, airbladder and muscle. The virus was recovered from all tissues examined. Kidney and spleen pools had the highest titer, reaching $10^{6.05}$ to $10^{6.90}$ TCID$_{50}$/ml.

Discussion

The virus isolated from cultured hirame and ayu appears to be a previously unreported pathogen of marine fish. No evidence of a similar agent is noted among recent listings of fish viruses.

Fish rhabdoviruses, such as IHNV, VHSV, SVCV, PFRV, EVA and EVX are well known. Swim bladder inflammation (SBI) virus proved to be morphologically and serologically identical to SVCV (Bachman and Ahne 1973, 1974). HRV was not neutralized with the antisera against IHNV, VHSV, SVCV, PFRV, EVA or EVX. Cross neutralization tests indicated that HRV was clearly distinguishable from these reference rhabdoviruses.

HRV shows IHNV-like CPE with eventual lysis of FHM, EPC, BF-2, RTG-2, STE-137, HF-1, BB, YNK, CCO and EK-1. However, the CHSE-214, CHH-1 and KO-6 cell lines, all with an epitheloid morphology and derived from salmonid fish, were refractive to HRV. The pattern of this cell susceptibility was clearly different from IHNV and VHSV, and distinguishable from the rhabdoviruses of cod ulcus syndrome (Jensen et al. 1979) or crab (Johnson 1984).

Although the rhabdoviruses isolated from warmwater fish (SVCV, SBIV, PFRV, EVA, EVX), the virus isolated from grass carp (Ahne 1975) and from a North American cichlid (Malsberger and Lautenslager 1980) replicate at 25°C or above, HRV did not replicate at 25°C. A comparison between HRV and *Rhabdovirus salmonis* (Osadchaya and Nakonechnaya 1981) could not be made because of the limited information available.

The pathogenicity and virulence of HRV for hirame was observed following intraperitoneal injection into 100 to 250 g fish (Gorie et al. 1986). The cumulative mortality was 20%, but mortality was not observed at 15°C or above. The mortality of hirame in natural outbreaks in Hyogo was 25% (1984) and 7.2% (1985). For Kagawa Prefecture it was 3.3% and greater than 90% in Hokkaido. The size of fish affected was 100 to 700 g. This high mortality among naturally infected hirame observed in Hokkaido may be caused by the lower rearing temperatures in the winter.

HRV was lethal for rainbow trout and the cumulative mortality was 60%. The signs of HRV infection in rainbow trout were similar to those of VHS, with hemorrhaging in muscles commonly seen. Histopathologically, hematopoietic cells were pyknotic or necrotic in hirame (Kimura et al., unpublished data). Castric and de Kinkelin (1984) have reported that VHSV is pathogenic for sea bass and turbot, both marine species. The gross signs and histopathological data reported indicate a condition similar to that of HRV-infected hirame. In this study, chum, coho and masu salmon and ayu fry did not experience high mortality when exposed to HRV by immersion method, but HRV was isolated from dead and surviving fish. A longer observation period may have resulted in a higher mortality.

Evidence suggests HRV is a new and pathogenic fish virus which is considered to be an important pathogen of cultured hirame and salmonids in Japan.

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Table 1. Biochemical and biophysical characterization of hirame rhabdovirus.

Characteristics	Log. TCID$_{50}$/ml	
	HRV 8401 H	HRV A-8401 H
Control	6.05	6.05
Ether treatment, 5°C, 18 h	<1.80	<1.80
pH 3.0, 3 hr	4.05	4.30
50°C, 2 min.	<0.80	<0.80
60°C, 1 min.	<0.80	<0.80
IUdR, 50 µg/ml*	6.05	6.30
Hemagglutination of human 0 type erythrocytes*	negative	negative

*See text.

Table 2. Cross neutralization test of the HRV and IHNV, VHSV, SVCV, EVA, EVX and PFRV.

Virus	Virus titer Log $TCID_{50}$ /well	Antisera (ND_{50})[f]									
		HRV	IHNV		VHSV		SVCV		PFRV	EVA	EVX
		8401-H	HV-1[a]	H[b]	K[c]	A[d]	H	K	K	H	H
HRV 8401-H	1.76	160	<17	<42	<42	<42	<42	<42	<17	<42	<42
IHNV HV-1	2.00	<17	90	ND[e]	ND	ND	ND	ND	ND	ND	ND
IHNV H	1.76	<17	ND	141	<42	<42	<42	<42	<17	<42	<42
SVCV H	2.00	<17	ND	<42	<42	<42	141	ND	<17	<42	<42
EVA H	1.55	<17	ND	<42	<42	ND	<42	<42	ND	≥ 281	≥ 281
PFRV K	2.25	<17	ND	<42	ND	ND	ND	ND	≥ 112	ND	ND

[a] Provided by the Ministry of Agriculture and Fisheries.
[b] Provided by Dr. B.J. Hill.
[c] Provided by Dr. P. de Kinkelin; antiserum against VHSV was polyvalent.
[d] Provided by Dr. W. Ahne.
[e] Not determined.
[f] ND_{50} = 50% neutralization titers against 100 $TCID_{50}$ virus.

Table 3. Comparison of cell line susceptibility to HRV and IHNV infection.

Cell line			Cell	HRV 8401H		IHNV ChAb	
Abbreviation	Source		morphology	CPE[1]	Titer[2]	CPE	Titer
RTG-2	Rainbow trout	Gonad	F[3]	+	6.30	+	4.30
EPC	Carp	Epithelioma	E[4]	+	7.30	+	6.30
FHM	Fathead minnow	Caudal trunk	F	+	6.80	+	5.80
BF-2	Blue gill	Fin	F	+	5.55	+	4.55
YNK	Yamabe	Kidney	F	+	6.30	+	4.55
STE-137	Steelhead trout	Embryo	E	+	5.05	+	4.80
CHSE-214	Chinook salmon	Embryo	E	–	<2.80	+	5.55
CHH-1	Chum salmon	Heart	E	–	≤3.05	+	6.30
KO-6	Kokanee salmon	Embryo	E	–	<2.80	+	4.80
HF-1	Hirame	Fin	E	+	5.80	–	<2.80
BB	Brown bullhead	Caudal trunk	F	+	5.30	–	<2.80
EK-1	Eel	Kidney	E	+	3.80	–	<2.80
CCO	Channel catfish	Ovary	F	+	4.55	–	<2.80

[1] Cytopathic effect.
[2] Log. $TCID_{50}$/ml.
[3] Fibroblast.
[4] Epitheloid.

Table 4. Comparison of mortality in rainbow trout, masu salmon, coho salmon, chum salmon and ayu artificially infected with HRV.

Species of fish	Method of infection	Experiment	Number of fish employed	died days after exposure 3	6	9	12	21	Cumulative mortality (%)
Rainbow trout (B.W. 8.0 g)	IP[1]	Test	10	0	1	2	3	0	60
		Control	10	0	1	0	0	0	10
Masu salmon (B.W. 6.0 g)	IP	Test	10	0	0	0	0	0	0
		Control	10	0	0	0	0	1	10
Coho salmon (B.W. 0.5 g)	IS[2]	Test	50	0	0	0	0	0	0
		Control	50	0	0	0	0	0	0
Chum salmon (B.W. 0.2 g)	IS	Test	50	1	3	1	0	0	10
		Control	50	1	1	0	0	1	6
Masu salmon (B.W. 0.2 g)	IS	Test	30	2	0	0	0	0	7
		Control	30	0	0	0	0	0	0
Ayu (B.W. 0.5 g)	IS	Test	15	0	0	0	0	0	0
		Control	15	0	0	0	0	0	0

[1] Intraperitoneal injection; exposing dose, 1.0×10^4 $TCID_{50}$/fish.
[2] Immersion method; exposing dose, 1.0×10^3 $TCID_{50}$/ml, 60 min. 10°C.

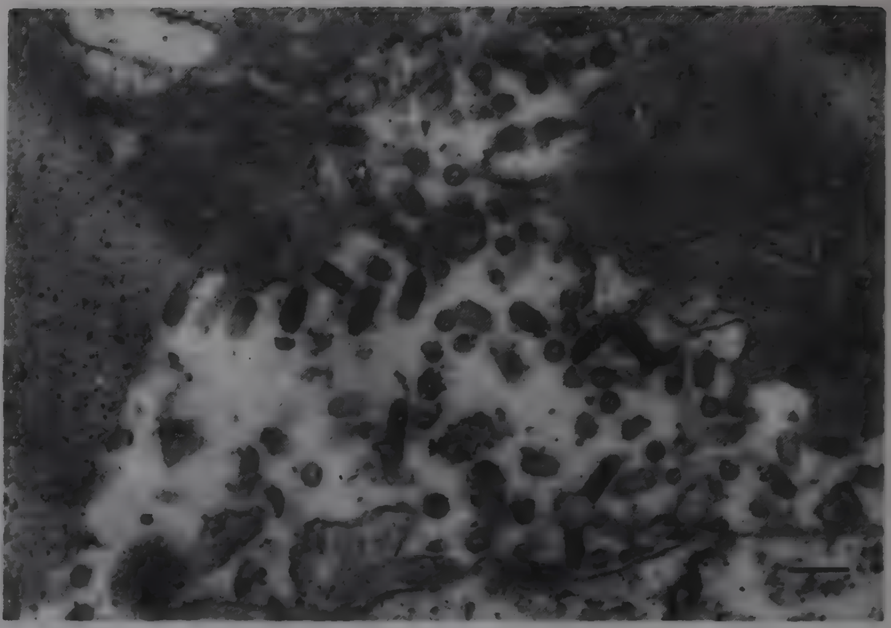

Fig. 1. Electron micrograph showing large numbers of bullet shaped virus particles in RTG-2 cell infected with HRV. Stained with 2% uranyl acetate, bar = 200 nm.

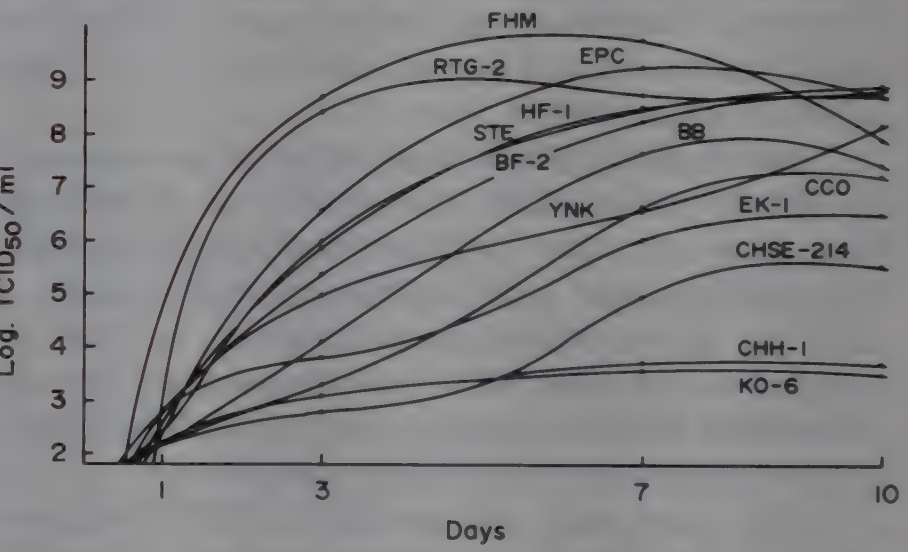

Fig. 2. Replication of HRV in selected fish cell lines incubated at 15°C, M.O.I. = 0.1.

Snakehead Fish Virus Isolation and Pathogenicity Studies

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Abstract

During the epizootic which occurred among the freshwater fish in Thailand in 1983 and 1984, the isolation of viruses was attempted. Various species of infected fish, e.g., snakehead fish *(Ophicephalus striatus)*, serpent fish *(Channa micropeltes)*, sand goby *(Oxyeleotris marmoratus)*, three-spot gourami *(Trichogaster trichopterus)*, striped croaking gourami *(Trichopsis vittatus)*, siamese fighting fish *(Betta splendens)* and wrestling half-beak *(Dermogenus pustillus)* were examined for viral infections. The fish showed macroscopic lesions, scale erosion and deep ulcers on all parts of their bodies. *Aeromonas hydrophila* was the main bacteria isolated, while fungi and parasites were also detected. A probable new virus, designated snakehead fish virus (SHV), was isolated. The virus was not affected by ether or chloroform and was resistant at 60°C for 30 minutes. SHV produced cytopathic effects, rounded cells and complete destruction of cell sheet, on BB, BF_2 and FHM cells. Experimental infectivity studies using snakehead fish showed that SHV elicited lesions similar to those seen in naturally affected fish. Cystoplasmic inclusion bodies were obvious and SHV was demonstrated in the membrane-bound body. The size of the virus is about 100 nm, comparable to that of IPN virus.

Introduction

In the winter of 1982, the first fish epizootic occurred in Thailand. The epizootic spread over a quarter of the country and caused heavy economic damage. The affected natural and cultured fish, mostly scaled freshwater fish, showed typical lesions of scale erosion and deep ulcers on all parts of their bodies. Snakehead fish *(Ophicephalus striatus)* was markedly infected. *Aeromonas hydrophila* could be isolated from ulcers and internal organs (Boonyaratpalin et al. 1983; Poonsuk et al. 1983) and are believed to be secondary invaders (Saitanu and Poonsuk 1984). *Epistylis* sp., a protozoan, and various other parasites were also demonstrated (Tangtrongpiros et al. 1983a; Tonguthai 1985). Likewise, virus-like particles showing a different morphology were also observed by electron microscope (Wattanavijarn et al. 1983; Jhuingsamarnyat et al. 1984). Tangtrongpiros et al. (1983b) observed the cytopathic effect on EPC cell line after inoculation with cell-free filtrate from the liver, kidney and spleen of diseased snakehead fish. Insecticides were also believed by some scientists to be the cause of the epizootic, which occurs annually during the cool months from October to February. The authors believe that the disease was not related to other environmental factors except low temperature and that the virus may play a major role in infection.

In order to elucidate the cause of disease, the isolation of viruses from affected fish was undertaken during 1983 and 1984. This report concerns the isolation, pathogenicity and physical properties of the snakehead fish virus (SHV).

Materials and Methods

Affected snakehead fish were collected during the outbreak in January to March 1983. Serpent fish *(Channa micropeltes)*, sand goby *(Oxyeleotris marmoratus)*, three-spot gourami *(Trichogaster trichopterus)*, striped croaking gourami *(Trichopsis vittatus)*, siamese fighting fish *(Betta splendens)* and wrestling half-beak *(Dermogenus pustillus)* were also collected during the outbreak in 1984. All samples were kept at -20°C.

Spleen, kidney and liver were taken from each fish. samples were ground with sterile sand in a mortar and pestle in 10 volumes of normal saline. The supernatant was collected and filtered through 0.22μm membrane filter. The cell-free filtrate was inoculated onto BF-2, BB, FHM cell lines in Eagle's MEM with 5% fetal bovine serum, 100 iu/ml penicillin, 100 μg/ml streptomycin, 100 μg/ml neomycin and 100 iu/ml mycostatin, in triplicates. The tubes were incubated at 25°C and examined for cytopathic effect (CPE) over 10 days. Tubes showing CPE were harvested and passed onto the corresponding cell lines.

The second passage of virus was kept at -20ºC for further studies.

The effect of ether and chloroform was determined (Christensen 1977). Heat tolerance was evaluated by keeping 5 ml of cultured virus at 60ºC for 30 min. After the exposure, the virus was titrated in BF-2 cells.

In Trial A, the third passage of SHV-1 in BB cells was used. Forty snakehead fish of 10-15 cm and 30-80 g were equally divided into two groups. One group of fish was intraperitoneally injected with 2 x 10^6 $TCID_{50}$ of virus. The other, as a control, was intraperitoneally injected with BB cells. The injected fish were separated and put in glass aquaria. Water was aerated and kept at 25-28ºC throughout the study. The fish were observed for 14 days and those developing lesions were subjected to histopathological and electron-microscopic examinations.

Trial B was undertaken as described in trial A with slight modification. A fourth passage of SHV-1 and larger fish (21-26 cm) and 100-250 g were used.

For histopathological studies, spleen, liver, muscle and intestine were fixed in 10% phosphate buffer formalin and processed according to conventional histological techniques. Paraffin blocks were cut at 5μm thickness and stained with hematoxylin and eosin.

Specimens were fixed with 4% glutaraldehyde in phosphate buffer at 7.4 pH for two hours and postfixed in 1% osmium tetroxide for an additional hour. They were then dehydrated in a series of graded alcohol, cleared with propylene oxide, and embedded in epon Epoxy. Semithin sections were cut using glass knives mounted on a Porter Blum MT-2 ultra microtome, and stained with toluidine blue (Trump et al. 1961) for light microscopic examination and area orientation. Ultra thin sections were also cut with glass knives and doubly stained with uranyl acetate (Watson 1958) and lead citrate (Venable and Coggeshall 1965). They were examined and photographed in a Hitachi H-300 elctron microscope operated at 75 KV.

Results

Viruses were isolated from most of the samples. The CPE in cell lines was observed 3-7 days post-inoculation. The infected cells were rounded and finally sloughed from the glass.

SHV-1, a tested strain, was not affected by ether or chloroform. The infectivity of virus in cell line was slightly decreased after 30 min. exposure to 60ºC.

In Trial A, 80% of injected fish developed disease with no mortality observed throughout the study. They exhibited pathology similar to the natural infection, i.e., scale erosion and slight ulceration, but no visible lesions in the internal organs. All control fish showed no clinical signs. In Trial B, neither injected nor control fish was infected.

The liver of infected fish showed congestion with no focal necrosis. The liver cells were swollen with granular cytoplasm. Vacuolar degeneration was observed in some liver cells. Acidophilic intracytoplasmic inclusion bodies, round to oval in shape, were detected in liver cells (Fig. 1). Most of the inclusion bodies were rimmed by a clear zone and were rather common in distribution. Pancreas, spleen, intestine and muscle showed no significant lesions. There was no histopathological change in tissues from fish of either trial B or the control.

Under the electron microscope, the virus particles were demonstrated in infected BF_2 cells (Fig. 2) and the hepatocytes of infected snakehead fish (Fig. 3).

Discussion

Results show that SHV is similar to infectious pancreatic necrosis virus in terms of physical properties including morphology and size range (Adair and Ferguson 1981). However, the infectivity of SHV in BF_2 and FHM is different from some strains of IPNV. Our isolated virus could produce CPE in both BF_2 and FHM in contrast to IPNV isolated from eels which failed to infect FHM (Hudson et al. 1981; Vestegard-Jorgensen and Kehler 1971), while the reference strain, ATCC VR 299 IPNV could replicate in this cell (Nicholson et al. 1979).

The infectivity of SHV probably depends on the number of passages and the age of experimental fish. The results were in agreement with other studies on the infectivity of IPNV (Franci and Savan 1971; Sano et al. 1981). Our histopathologic and electron microscopic examinations showed similar figures to the natural infection (Ruttanaphani et al. 1983). Therefore, SHV may be considered as the primary cause of the disease. In order to elucidate the role of SHV in the epizootic, further pathogenicity studies should be undertaken under various conditions similar to those that occur during the natural outbreak, e.g., low temperature and using different kinds of fish. Likewise, comparison of the serological relationship of SHV with the other strains of IPNV is needed.

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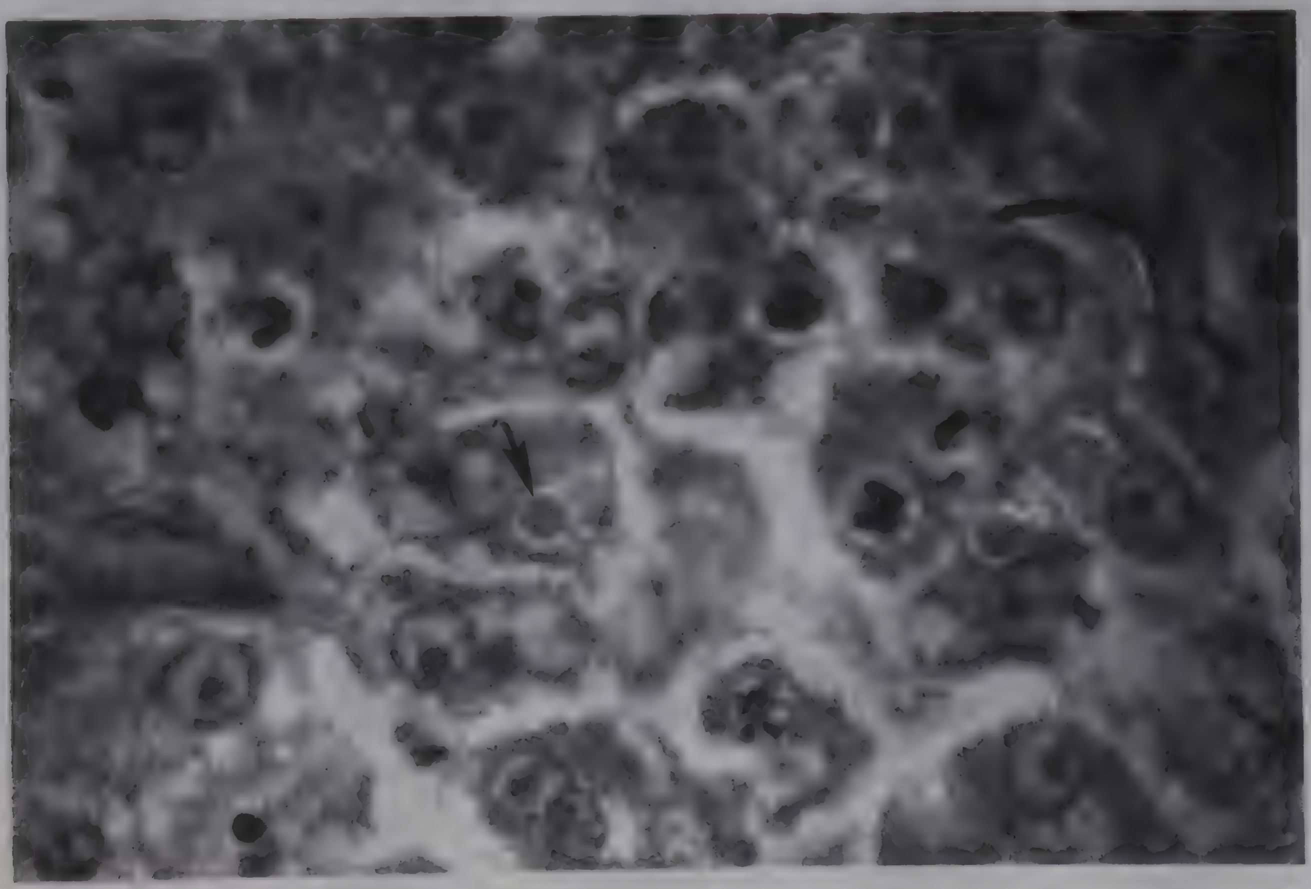

Fig. 1. Intracytophasmic inclusion bodies (arrows) in liver cells of snakehead fish experimentally infected with SHV. (H & E x 1,000).

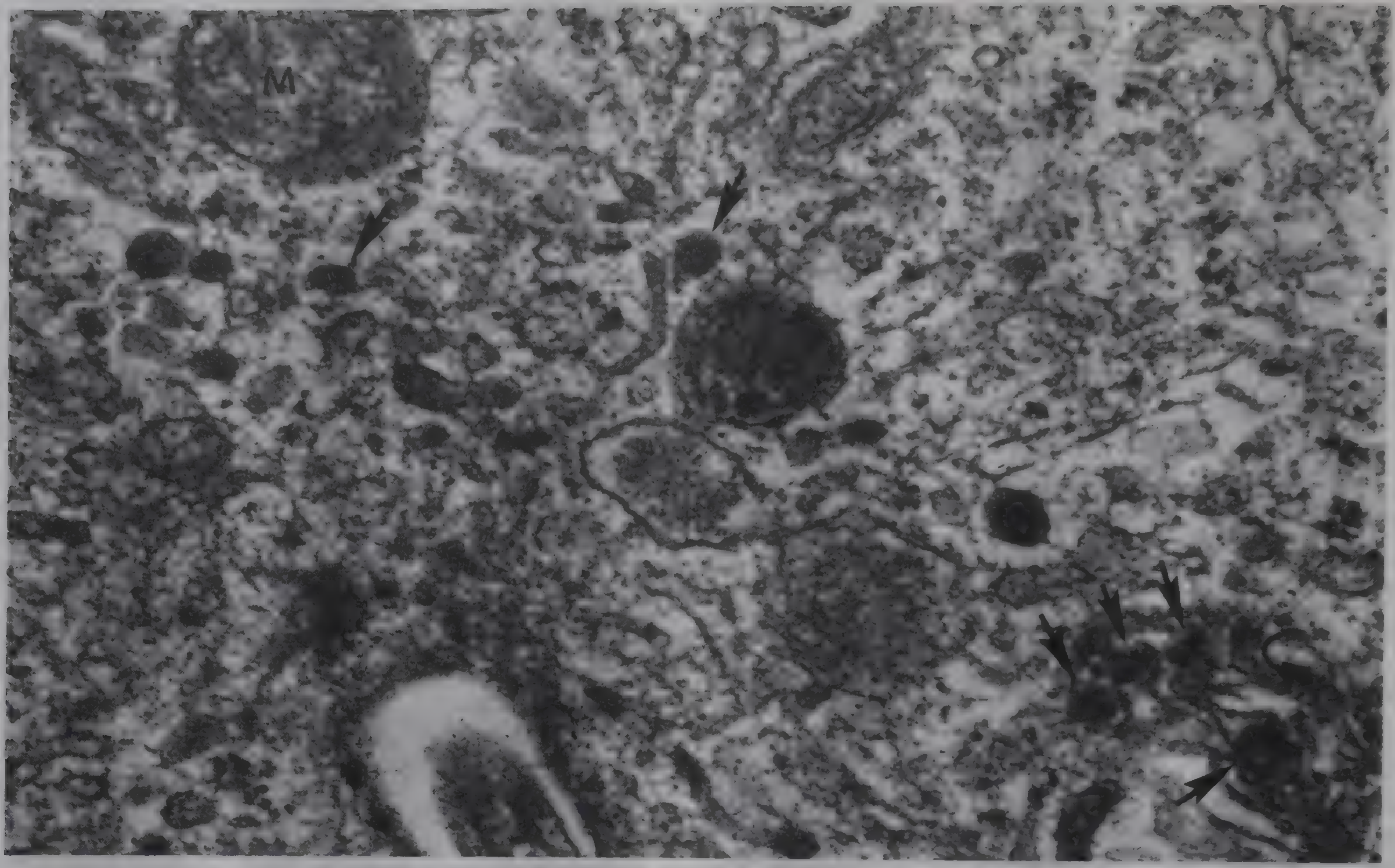

Fig. 2. BF_2 cells inoculated with SHV. Virus particles (arrows) with average diameter of 100 nm are seen in the cytoplasm of this culture cell. Some are seen within the cisternae of rough endoplasmic recticulum. (M, mitochondria; x 47,500).

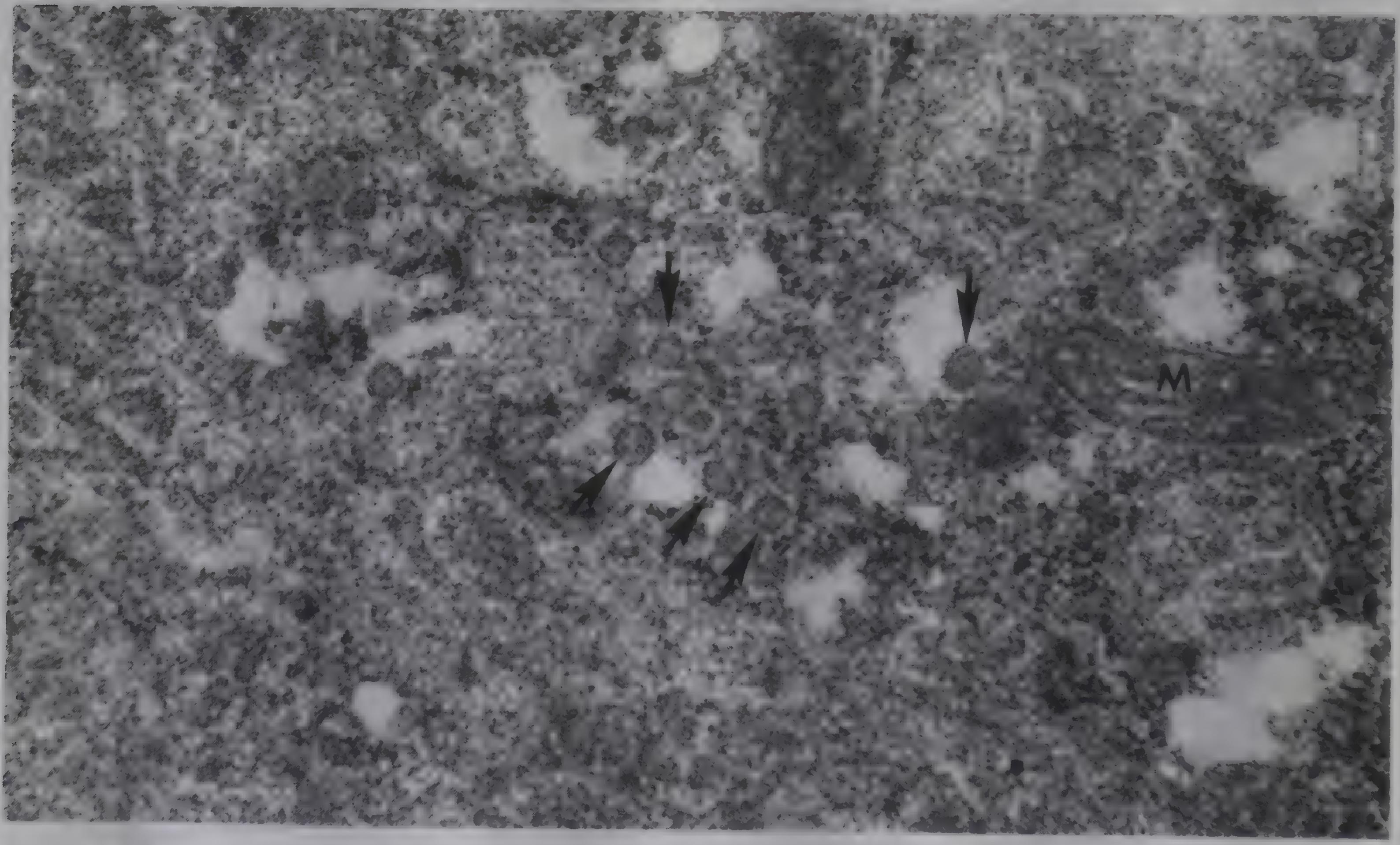

Fig. 3. An electron micrograph of a portion of snakehead fish hepatocyte showing several spherical virus particles (arrows) within its cytoplasm. The particle size ranges from 70 to 120 nm in diameter. (M, mitochondria; x 35,000).

Septicemias of Marine Crabs and Shrimp

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Abstract

Septicemic conditions were associated with several illnesses of high mortality and prevalence of infection among marine crabs and shrimp farmed or imported into Singapore. "Orange crab" disease was responsible for the loss of approximately 200 kg of mangrove crab *(Scylla serrata)* cultured in floating net cages within two weeks. Aseptic culture of crab hemolymph yielded variously *Vibrio alginolyticus, V. fluvialis, V. parahaemolyticus, Bacterionema* sp. and *Alteromonas* sp. "White pleura" disease was the most commonly diagnosed disease of *Penaeus indicus* and *P. merguiensis* in the postlarval and grow-out phase. Aseptic culture of hemolymph from diseased prawns consistently yielded gram-positive cocci arranged in tetrads. These were identified by biochemical tests as *Micrococcus* sp. "Red vein" disease of *P. monodon* postlarvae is a major cause of loss for commercial hatcheries. Microscopic examination revealed the presence of large numbers of unidentified red-pigmented eukaryotic organisms, filling the entire ventral sinus and hemolymph spaces extending into the appendages. Moderate mortalities and viral infection among newly-imported spiny lobster *(Panulirus* sp.*)* were linked to *V. harveyi* septicemia.

Introduction

Wyatt (1975) estimated that invertebrate blood or hemolymph constitutes 25 to 40% of an insect's body weight. Crustacean hemolymph probably occupies the same proportion because both phyla utilize similar open, diffusive circulation systems. Adverse changes in such a large organ must have a significant effect on an animal's health. Blood poisoning or septicemia is characterized by a severe bacteremic infection, generally involving the significant invasion of the bloodstream by microorganisms from a focus or foci in the tissues and possibly the multiplication of microorganisms in the blood. Gaffkemia of lobsters is a classic crustacean septicemia, caused by the gram-positive bacterium *Aerococcus viridans* (var.) *homari*. This propensity to leak into or proliferate in the circulatory system is not confined to bacteria. "Grey crab" disease is caused by systemic infection with the ameboflagellate *Paramoeba perniciosa* (Sprague and Beckett 1968), while dinoflagellates of the genus *Haematodinium* have been reported (Newman and Johnson 1975) in septicemia resulting in losses of blue crabs.

Bacteriological analysis requires aseptic isolation, whether from healthy or diseased animals. The only crustacean organ easily accessible to aseptic isolation is hemolymph. During routine postmortem analyses of crustacean diseases, several septicemic conditions were encountered.

Materials and Methods

Sterile, disposible tuberculin-type syringes with fitted 26-gauge hypodermic needles were used to isolate crustacean hemolymph. Live animals were anesthetized for assay by brief immersion in approximately 10°C cold seawater. Body areas for sampling were sterilized with 95% alcohol, applied for approximately 20 sec. Crabs were penetrated through the integument between the propodus and carpus or alternatively between the propodus and dactylus of the chela. Shrimp hemolymph was withdrawn from the ventral sinus by inserting a needle in an anterior direction at an angle of 5° to a depth of < 2 mm. Lobster hemolymph was similarly withdrawn from the dorsal sinus. Depending on the size of animal, between 0.01 and 0.1 ml were withdrawn each time for bacterial isolation or microscopic examination.

Standard bacteriological media were used for isolation: nutrient broth, blood agar, thiosulphate citrate bile salts (TCBS) agar, trypticase soy agar (TSA) and trypticase soy/0.1% NaCl agar. Biochemical identification of bacteria was carried out by Bacteriology Unit I, Central Veterinary Laboratory, Primary Production Department, using standard reagents. Antibiotic sensitivity tests were performed by the multidisk method.

Results and Discussion

"Orange crab" is an acute disease of mangrove crab *(Scylla serrata)* with moribund crabs displaying orange discoloration of the ventral carapace and pincers. In this case study, crabs weighing 300-600 g were caught from the wild, imported into Singapore, and stocked in floating net cages. During a two-week fattening period, almost 100% morbidity and 70% mortality, equivalent to a loss of approximately 200 kg, occurred. Dissection of acutely sick crabs revealed copious milky-white hemolymph. Microscopic examination demonstrated very high concentrations of gram-negative bacteria of *Vibrio* spp. Bacterial culture of hemolymph from diseased crabs yielded predominantly *Vibrio alginolyticus*. Aseptic culture of hemolymph taken from clinically healthy crabs or crabs in the non-acute phase of the disease, i.e., still with clear hemolymph, yielded variously *V. fluvialis*, *V. parahaemolyticus*, *Bacterionema* sp. and *Alteromonas* sp.

Based on *in vitro* antibiotic sensitivity tests on the two strains of *V. alginolyticus* and one strain of *V. parahaemolyticus* isolated from milky hemolymph, the farmer was advised to treat crabs with oxytetracycline or sulphadimethoxine-trimethoprim. Both drugs were administered at 0.1 g active ingredients per kg chopped trash fish, using fish mucillage and tissue exudate to bind the drugs. The farmer reported fewer losses in net cages receiving either treatment, compared to untreated net cages, although this report was not confirmed.

Although a clear septicemic condition was evident in moribund crabs, possibly causing death, the diversity of bacterial types suggests a fundamental weakening of normal immunity. This could have been due to previous infection by a primary pathogen. Certainly, the transmission of diseases through this crab consignment is through healthy crabs observed to cannibalize moribund crabs. It is possible that this facilitates the spread of pathogens, whether primary or secondary.

"White pleura" disease was previously reported by AQUACOP (1977). This was found in this study to be the most commonly-diagnosed disease of *Penaeus indicus* and *P. merguiensis* in the postlarval and grow-out stages. Gradual lethargy, anorexia and moderate mortalities were the first indications. White patches, presumably due to cellular accumulations, were seen on the pleura and subshell membranes of the rostrum and swimmerets. The severity of outbreaks was variable but in the acute phase, most shrimp would display anorexia and about 5 to 10% would develop white spots and die. In contrast to AQUACOP, who implicated *V. alginolyticus* in the etiology of this disease, in this study, aseptic culture of hemolymph from diseased shrimp consistently yielded gram-positive cocci arranged in tetrads. These were identified by biochemical tests as *Micrococcus* sp. The specific involvement of *Micrococcus* sp. in "white pleura" disease is yet to be proven but from the relatively high recovery rate, its pathogenicity for shrimp is considerably less than, say, of *A. viridans* for lobster. *In vitro* tests indicate that shrimp strains of *Micrococcus* sp. are sensitive to penicillin, the tetracyclines, chloramphenicol, streptomycin, neomycin, bacitracin, gentamicin and novobiocin but are significantly resistant to sulphonamides, nitrofurans and kanamycin. Given that natural recovery starts soon after the disease is noticed, therapy is best given as a prophylactic to postlarvae.

"Red vein" disease is a major cause of loss of *Penaeus monodon* postlarvae in commercial hatcheries. Nearly 100% mortalities are the rule. Sick shrimp display a continuous red vein on the ventral surface along the nerve chord. At night, such shrimp are reportedly luminescent. Microscopic examination shows complete occlusion of the entire ventral sinus and hemolymph spaces with a red-pigmented substance, suggesting a septicemia. Aseptic sampling of hemolymph was not possible due to the small size of *P. monodon* postlarvae. Tissue squashes, stained with lacto-phenol blue, revealed the presence of large numbers of freely-suspended eukaryotic cells. These could be a novel, red-pigmented eukaryotic pathogen or shrimp phagocytes that had consumed large amounts of red-pigmented bacteria. However, the disease was not susceptible to treatment with a wide range of antibiotics, suggesting a non-bacterial origin.

Vibrio harveyi septicemia was encountered in one batch of recently imported spiny lobster, *Panulirus* sp. Brown patches were observed on the soft integument between the cephalothorax and first abdominal segment. Moderate mortalities were sustained but the infection did not spread to other stocks. Lobster hepatopancreases were sent for histopathological examination, which revealed the presence of herpersvirus-like inclusion bodies. It is therefore not possible to say, in the absence of like cases, if viral infection or *V. harveyi* septicemia was the primary cause of lobster disease.

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Antibiotic Uptake of the Giant Freshwater Prawn *Macrobrachium rosenbergii* by the Osmotic Infiltration Technique*

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Abstract

Erythromycin and oxytetracycline uptake by the giant freshwater prawn, *Macrobrachium rosenbergii*, was evaluated by the two-step osmotic infiltration technique. Test prawns were immersed in 7.5% NaCl conditioning (hyperosmotic) solution for 30 min. then transferred to a freshwater (hypo-osmotic) solution containing 10 μg/ml of antibiotic. Antibiotic uptake was determined in test and control prawns at 5, 10, 20 and 40 min. Maximum concentration of erythromycin was observed in test prawns 10 min. after exposure, whereas the same concentration was not achieved in control prawns until 40 min. Oxytetracycline uptake was similar in treated and control prawns in that maximum tissue levels were observed after 40 min. in both cases. The uptake of erythromycin was significantly greater ($p < 0.05$) in prawns treated for 10 min. than in prawn treated for 5, 20 and 40 min. Osmotic infiltration did not significantly enhance oxytetracycline uptake ($p > 0.05$) at all time intervals tested.

Introduction

Fish populations maintained under crowded conditions inherent to aquaculture are subject to a variety of infectious diseases (Hanson and Goodwin 1977). Sometimes these diseases develop into epizootics, causing widespread mortality. Some of the more frequently observed agents involved in fish diseases are listed by Sindermann (1970) and Johnson (1977). The economic effect of diseases due to these agents is often devastating.

Although antibiotic therapy has been widely used for animal diseases, the value of the technique in controlling fish and shellfish diseases has not been fully exploited because large quantities of certain drugs are sometimes needed to bring about therapeutic concentrations in tissues and body fluid. Because of the similarity of physiological principles involved, optimum conditions for administration of antibiotics may be similar to those developed for administration of immunizing agents (vaccines) in fish. Incorporation of antibiotics into the feed often presents practical difficulties because the effectiveness of drugs is sometimes diminished during digestion and uniformity of dosage cannot be attained. Diseased fish are anorexic and tend not to eat the medicated diet (Roberts 1978). Parenteral administration (interperitoneal and intramuscular injection) has had limited use because of the stress and logistics associated with handling large numbers of relatively small individuals. Moreover, the method is labor-intensive and is restricted by the size of the fish being treated.

Osmotic infiltration has been successfully used as a method of administering vaccines to fish (Amend 1976; Antipa and Amend 1977; Croy and Amend 1977; Alexander et al. 1981; Bowers and Alexander 1981). Because large numbers of aquatic animals of any size could be treated simultaneously, the technique would appear to possess considerable practical value in administering antibiotics or therapeutic drugs to fish.

This study was designed to evaluate the use of the osmotic infiltration technique in administering antibiotics to the giant freshwater prawn, *Macrobrachium rosenbergii*.

Materials and Methods

Adult giant freshwater prawns 6 months old, weighing 18-40 g, reared at the Texas A&M University Aquaculture Research Center ponds were used in this study. During the holding period, the prawns were fed with Ralston Purina Experimental Marine Ration No. 25 (Ralston Purina, Checkerboard Square, St. Louis, Missouri).

The concentration of the conditioning solution was established by assessing the tolerance of prawns to sodium chloride (NaCl) in solutions containing 5, 7.5, 10 and 12.5% NaCl maintenance water representing 1,630, 2,445, 3,180 and 3,710 mosM, respectively. Osmotic pressure of the solutions was estimated on the basis of freezing point depression using an osmometer. Five animals were tested in each concentration for 20 min. after which they were returned to 0.5% NaCl (104 mosM) solution and observed for 30 min. Mortalities were recorded and behavior indicative of stress observed.

The two-step osmotic infiltration technique of Croy and Amend (1977) was used in the antibiotic uptake studies. Prawns were held at 28°C in maintenance water 48 hours prior to the test. Test animals were held in conditioning solution (7.5% NaCl) for 30 min., then transferred to freshwater containing the antibiotic at a concentration of 10 μg/ml. Samples from the abdominal muscles taken at 5, 10, 20 and 40 min. were weighed and diluted on a gram per volume basis using 0.1 M phosphate buffer 3 pH 8 for erythromycin and 0.1 M phosphate buffer 4 pH 4.5 for oxytetracycline. Each sample (12.5 ± 3.5 g) was thoroughly homogenized in a high speed blender for 5 min. The resulting suspension was centrifuged at 2,500 x g for 30 min. and the supernatant retained for the assay. Control prawns, which were not immersed in the conditioning solution, were processed in the same manner. Three replicates were made for the experimental and control animals, using one prawn for each replicate. Antibiotic uptake was assessed by the microbiological assay technique of Grove and Randall (1955) and that of the Association of Official Analytical Chemists (1975). Significant differences between the means of the control and the experimental animals at each time interval were determined with the t-test (Snedecor and Cochran 1980).

The antibiotics erythromycin (Eli Lilly Co., Indianapolis, Indiana) and oxytetracycline (Bristol Laboratories, Syracuse, New York) were used for the assay solutions and the preparation of standard response lines. *Sarcina lutea* ATCC 9341 was used to assay the antibiotic erythromycin and *Bacillus cereus* ATCC 11778 for oxytetracycline. Antibiotic medium 1 (Difco Laboratories, Detroit, Michigan) was used as the maintenance medium and for the preparation of inoculum and assay medium for both organisms.

Results

Result of the tolerance test of *M. rosenbergii* to the different NaCl concentrations are shown in Table 1.

Immediately after exposure to 12.5% NaCl (3,710 mosM), the prawns showed erratic and violent movement followed by quiescence. One prawn displayed an inverted position. Mortality did not occur during the 20 min. the prawns were exposed to this concentration, but 60% died when returned to freshwater. The animals which survived were lethargic. Although mortality was not observed in prawns exposed to 10% NaCl (3,180 mosM), the animals showed signs of stress after they were returned to freshwater. All the animals preferred to stay at the surface. The highest concentration tolerated by the prawns without apparent signs of stress or mortalities was 7.5% NaCl (2,445 mosM). This concentration was therefore used as the conditioning (hyperosmotic) solution.

Erythromycin and oxytetracycline concentrations in the abdominal muscles in treated and control prawns at different exposure times are shown in Figs. 1 and 2, respectively. The maximum concentration of erythromycin in treated prawns was 0.185μg/g tissue at 10 min. exposure time. Uptake in control prawns had a maximum concentration of 0.185 g/g tissue at 40 min. A significant difference ($p < 0.05$) was observed between the treated and control prawns at 10 min. Oxytetracycline uptake was high at 10 min. in treated prawns with a mean concentration of 0.099μg/g tissue, but the highest was at 40 min. (0.100 μg/g tissue). A steady absorption rate was observed in control prawns, giving a mean concentration value of 0.070μg/g tissue for 5, 10 and 20 min. exposure times. This value increased to 0.090μg/g tissue after 40 min. No significant difference ($p > 0.05$) was observed between the treated and control prawns at all time intervals tested.

Discussion

The osmotic infiltration technique employed in this study involved initial immersion in a hyperosmotic solution followed by immersion in a hypo-osmotic solution containing the antibiotic. The first step has a dehydrating effect on the membranes of the animals (Amend and Fender 1976). Water is lost as it passes through a semi-permeable membrane from an area of high to low water concentration (Lockwood 1963; Prosser 1973; Hill 1976). When the animals are returned to a hypo-osmotic solution after a period of dehydration, the antibiotic is absorbed when water is taken up until the "optimum" osmotic concentration for the particular animal is achieved (Prosser 1973).

Amend and Fender (1976) proposed that the primary route of entry of bovine serum albumin (BSA) during hyperosmotic infiltration (HI) is through the lateral line of fish. However, Bowers and Alexander (1981) demonstrated that the point of entry of bacteria during HI was primarily through the gills and not through the lateral line. This is because the gills are structurally weak and are the most vulnerable sites for entry of any foreign substance during osmotic stress. This observation was again demonstrated and confirmed by Alexander et al. (1981). According to Schoffeniels and Gilles (1970), the mechanisms responsible for the active uptake of salts appear to be located mainly in the gills among crustaceans. The structure of the gill membrane makes it permeable to many solutes. While uptake of antibiotic in *M. rosenbergii* may have also been through the gills, the exact route of entry is not known.

Techniques for mass delivery of antibiotics should be practical, efficient, quick and cheap, not limited by the size or number of fish that can be handled, and must not be stressful to the fish. Results of this study indicate that osmotic infiltration may not be applicable to all types of antibiotics or drugs. While erythromycin uptake during HI was beneficial at 10-min. exposure, the technique was not beneficial as far as the antibiotic oxytetracycline was concerned. Therapeutics have to be administered over a certain period of time at regular intervals. Because of the stress involved in following the regimen in this study, the value of omostic infiltration in administering drugs or antibiotics may be limited.

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Table 1. Tolerance of *M. rosenbergii* to various NaCl concentrations at five animals/concentration.

[NaCl] %	Osmotic Conc. (mosM)	Survival n	Survival %
5	1,630	5	100
7.5	2,445	5	100
10	3,180	5	100
12.5	3,710	2	40

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*SEAFDEC Contribution No. 207

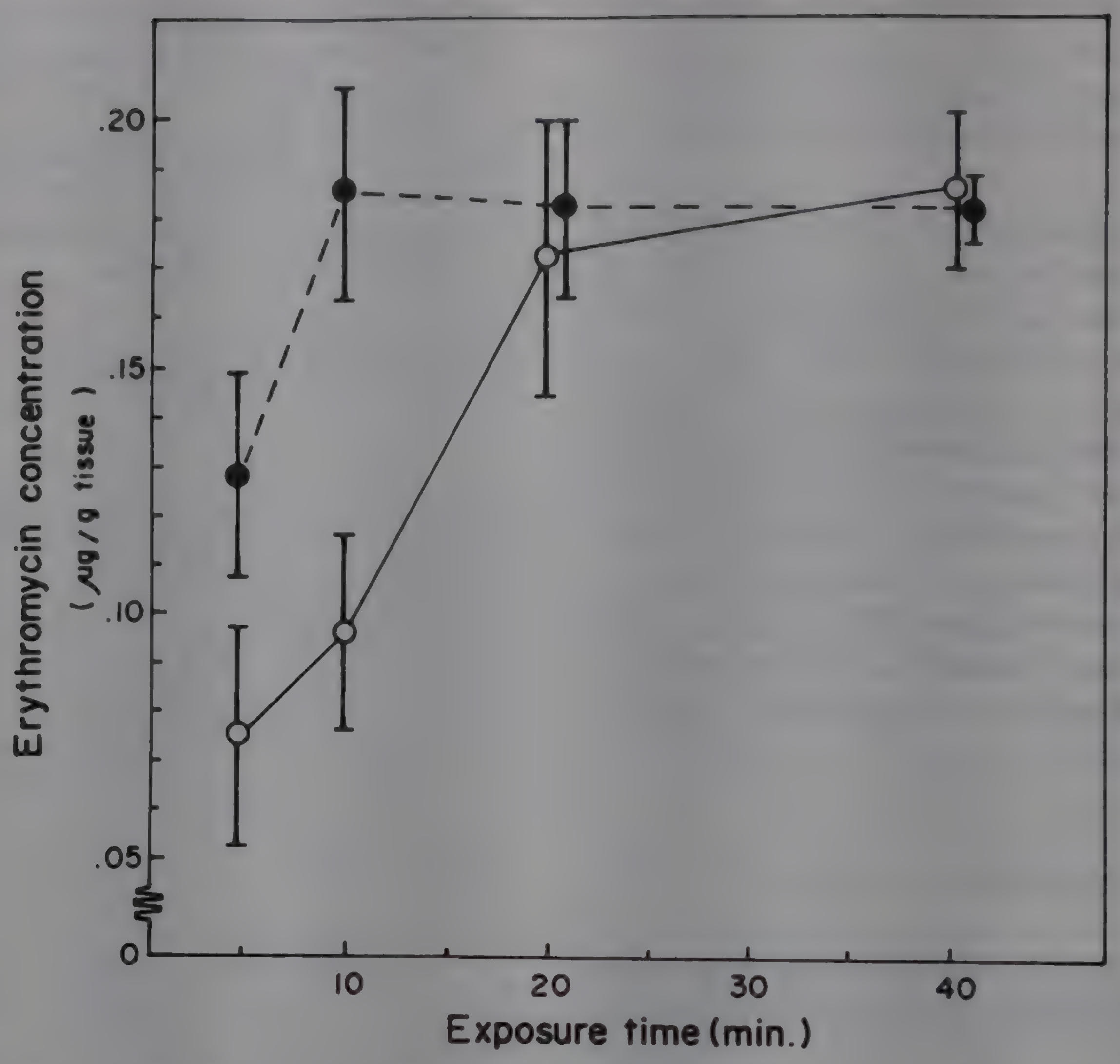

Fig. 1. Erythromycin concentration (µg/g tissue) in treated (— — — —) and control (———) shrimp at 5, 10, 20 and 40 min. exposure time. Each point represents the mean of three replicates, while the vertical bars represent the standard deviation.

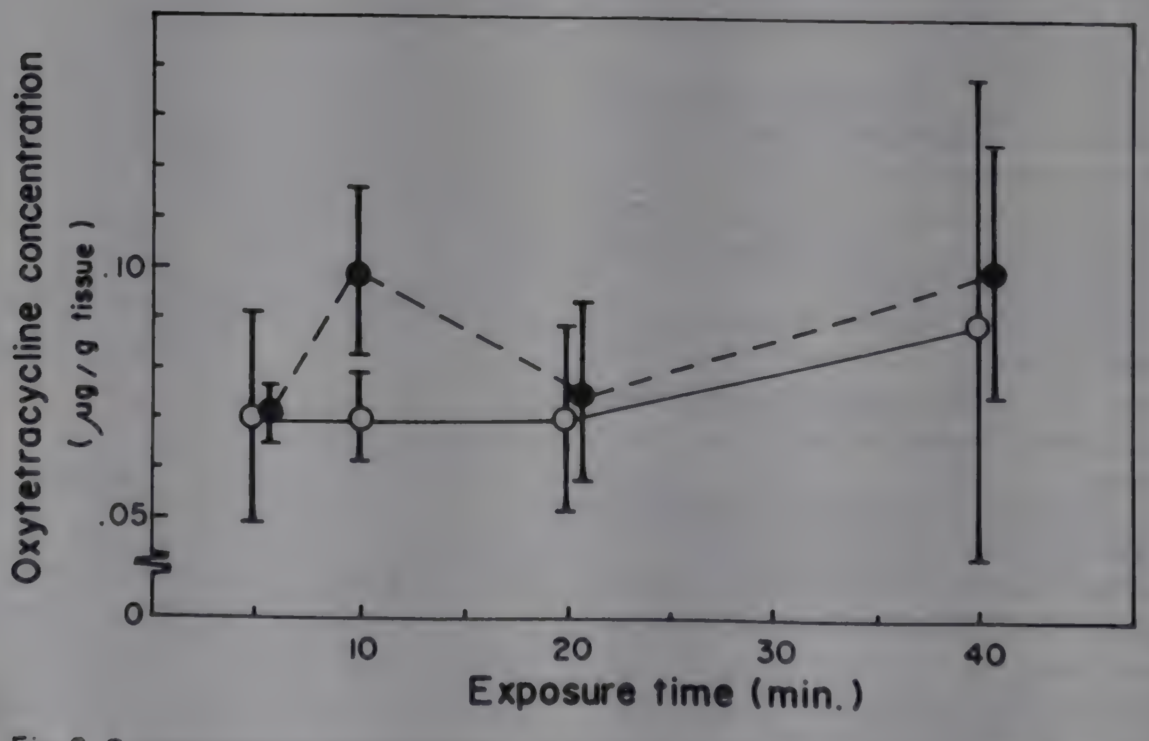

Fig. 2. Oxytetracycline concentration (µg/g tissue) in treated (— — — —) and control (———) shrimp at 5, 10, 20 and 40 min. exposure time. Each point represents the mean of three replicates, while the vertical bars represent the standard deviation.

Possible Cause of Mortality of *Oreochromis mossambicus* Eggs Under Artificial Incubation

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Abstract

Artificial incubation of tilapia eggs is of increasing interest among aquaculturists. In 1983, in one of the experimental artificial incubation systems at the University of Stirling, considerable egg mortalities were encountered. The physicochemical and microbiological qualities of hatchery water such as dissolved oxygen, pH, temperature, total ammonia, un-ionized ammonia, total nitrite, total viable heterotrophic bacteria (TVHB), total viable fungal spores (TVFS) and total viable Saprolegniaceae spores (TVSS) were monitored closely. All the physicochemial qualities measured were well below the lethal levels for the developing eggs. The TVFS fluctuated between 10,000 and 35,000 spores/l and the TVSS increased from 700 to 900 spores/l over eight weeks. The hatchability of viable eggs was never above 67.2%, with a minimum of 47.2%. The pathogenic bacterial flora consisted of members of the genera *Aeromonas, Pseudomonas, Flavobacterium, Bacillus* and *Micrococcus*. The potentially pathogenic fungal flora consisted of species of *Saprolegnia, Achlya, Pythium, Fusarium* and *Allomyces*. Scanning electron microscopic studies were carried out on the incubating eggs. The major cause of egg mortality was found to be of bacterial origin, initiated by mechanical injuries to the egg surface. A hypothetical mechanism is discussed.

Introduction

The importance of a constant supply of quality fry in tilapia culture has generated considerable interest in the hatchery rearing of tilapia eggs and early fry. This has led to experiments on the development of various types of artificial systems which simulate special conditions characterizing oral incubation. Various researchers have demonstrated such systems, most of which utilize recirculated water with varying success (Shaw and Aronson 1954; Rothbard and Pruginin 1975; Valenti 1975; Lee 1979; Rothbard and Hulata 1980; Rana 1986).

The detrimental effects of water reuse on artificially-reared fish have been well documented (Burrows 1964; Larmoyeux and Piper 1973; Mayer and Kramer 1973). During water reuse, the metabolic activities of the fish reduce the dissolved oxygen content and metabolic products are added to the water. Reduction of oxygen can be overcome by aeration but metabolic products, especially nitrogenous compounds, continue to accumulate with deleterious result to the fish, eggs and fry.

Artificial incubation of *Oreochromis* eggs has been carried out in the Institute of Aquaculture, University of Stirling for several years. In early 1983, heavy egg mortalities were observed in one of the experimental recirculatory incubation systems. Preliminary investigations indicated that the heavy mortalities may have been caused by reduced water quality. This experiment was therefore designed to examine and monitor the physical, chemical and microbiological qualities of hatchery water to understand the possible causes and mechanisms of egg mortality under artificial hatchery conditions.

Materials and Methods

Eggs were incubated in cylindrical plastic jars with rounded bottoms. A series of six jars were placed in a single 12-l transparent perspex aquarium, eight such aquaria comprised the system. Water was gravity fed from a 250-l header tank into the incubation jars. The water flow into the incubators was controlled to ensure gentle agitation of the eggs. The overflow water was collected in a 125-l settling tank partially filled with plastic biological filter rings, via a common drain pipe. The settling tank was coupled to a 125-l sump tank and the water was pumped back into the header tank by means of a power pump unit. The overflow water from the header tank was collected in the sump tank through a trickle-feed, gravel biological filtration unit. A temperature of 27 ± 1°C was maintained by thermostatically-controlled immersible heaters placed in the header tank. Small volumes of water were added periodically to compensate for water loss through evaporation.

Genetically pure *O. mossambicus* (as identified by McAndrew and Mujumdar 1983) were used throughout the study. The individually-tagged broodstock were maintained in a series of square fiberglass spawning tanks supplied with recirculated water of good quality. They were stocked at a ratio of three females to one male.

The carrying females were individually netted out between 12 and 24 hours postspawning. The egg clutches were removed with extreme care, opening the mouth of the fish under water so that handling would not damage the eggs. Prior to artificial incubation, the number of eggs in each clutch was estimated. A sample of 50 eggs from each clutch was preserved in Bouins' fixative to determine fertility rates.

Two to four groups of 100 eggs each from a single clutch were randomly sampled depending on the clutch size and were incubated separately. The remainder of the clutch was also incubated in the same system and used for the scanning electron microscopic studies. Incubation generally took four days. Once the eggs were completely hatched, the number of hatched fry were counted within 12 hours. Fry were left in the incubators for another eight days then discarded. No food was given during this period. The process was repeated for 51 days, during which 17 clutches of eggs were incubated, thus simulating the normal cycle of events in a small-scale commercial hatchery over seven weeks.

During the investigation, dissolved oxygen (DO) and pH of the hatchery water were measured daily with an oxygen meter (YSI Model 57) and a pH meter (Clandon Scientific). The total ammonia-nitrogen (NH_3-N) and total nitrite-nitrogen (NO_2-N) were also measured at three-day intervals according to Stirling (1985).

Total viable heterotrophic bacteria (TVHB) numbers in the water were estimated with the spread plate technique on Trypton Soya Agar (TSA) at three-day intervals. The method described by Iqbal and Webster (1973) was modified and used to estimate total viable fungal spore (TVFS) in the water. Total viable Saprolegniaceae spores (TVSS) were estimated using the methods described by Willoughby and Pickering (1977). The potentially pathogenic bacteria were isolated and identified from the hatchery water using the standard procedures described by Cowan and Steel (1974) and Frerichs (1984). The potentially pathogenic fungi were also isolated and identified using standard monographs.

Samples of ten eggs were removed from each clutch after 12, 24, 48 and 72 hours of artificial incubation, fixed in cacodylate buffered osmium tetroxide through an alcohol series, dried in a critical point and coated with gold/paladium mixture in a sputter coater. The eggs were then examined with an ISI 600 scanning electron microscope.

Results

Table 1 summarizes the pattern of change of the physical, chemical and microbiological qualities of the hatchery water during the investigation. The physical and chemical qualities changed only slightly. NH_3-N and NO_2-N remained low and were still less than 0.5 mg/l and 0.03 mg/l, respectively, at the end of the experiment.

In contrast, all microbiological parameters showed a gradual increase over the experimental period (Fig. 1a). TVHB increased from 1.41×10^3/ml to 4.49×10^4/ml while TVFS showed an approximately threefold increase from 11,800 spores/l to 35,250 spores/l. The most dramatic increase, however, was TVSS which incresed from 750 to 9,000 spores/l, an increment of approximately 12-fold.

The percentage hatchability of the fertilized eggs was never above 67.2 with a minimum of 47.2 and fluctuated irregularly during the investigation (Fig. 1b).

The potentially pathogenic bacteria consisted of two species of *Aeromonas*, including *A. hydrophila*, three species of *Pseudomonas*, including *P. fluorescens*, *Flavobacterium* sp. *Chromobacterium violaceum*, *Micrococcus* sp., and *Bacillus* sp. Pathogenic fungi consisted of *Saprolegnia* sp., three species of *Achlya*, including *A. prolifera* and *A. flagellata*, *Pythium* sp., two species of *Fusarium* and *Allomyces* sp. The major contributor to the large numbers of TVFS was *Trichoderma* sp. which was also abundant in the air.

Scanning electron micrographs of the eggs 12 hours postincubation showed rupture of the chorionic membrane and herniation of the yolk sac. Deposition of foreign material was evident on the ruptured and/or other areas of the egg surface. The foreign material appeared to consist of organic matter, probably debris. By 24 hours postincubation, localized bacterial colonies were evident on the egg surface, mainly centered around foci of organic debris. Between 24 and 36 hours postincubation, the localized bacteria spread over larger areas, almost covering the entire surface of some of the eggs. Samples examined 48 hours postincubation clearly showed fungal mycelial development on the egg surfaces already covered with bacteria. Fungal hyphae were found on the eggs only in association with bacteria.

Discussion

The results clearly indicate that the physicochemical quality of the hatchery water was not greatly reduced after continuous incubation of 17 clutches of *O. mossambicus* eggs in 51 days. Despite the fact that approximately 50% viable egg mortality occurred, resulting in the accumulation of considerable amounts of nitrogenous material in the water, there was relatively little increase in NH_3-N and NO_2-N in the hatchery water. This indicates a high efficiency of the biological filter rings in maintaining a high chemical quality of reused water. Spotte (1979) defined systems of this nature as "conditioned systems" and described them as "ones in which the nitrifying bacteria are in dynamic equilibrium with routine formation of their energy sources".

It has previously been found that eggs and early yolk sac fry were generally less susceptible than adults to adverse physical and chemical qualities of the water, including ammonia and nitrite (Rice and Stokes 1975; Holt and Arnold 1983). Fish eggs and sac fry stages are also reported to possess a certain degree of resistance to various toxicants such as endrin and DDT compared to adults (Burdick et al. 1964; Wenger 1973). Holt and Arnold (1983) reported that the hatchability of red drum, *Sciaenops ocellatus*, eggs was 88-97% at un-ionized ammonia levels as high as 7.2 mg/l while 100 mg/l NO_2-N did not significantly increase egg mortality. Although there are no published reports on the tolerance levels of *O. mossambicus* eggs to NH_3-N or NO_2-N, investigations carried out in this institute clearly indicate that their tolerance levels are well above the observed levels in the incubation system (Subasinghe, unpublished data). The observed egg mortalities were therefore unlikely to be related to the physical and chemical qualities of the hatchery water.

The behavior of the heterotrophic bacterial populations in recirculated water systems for fish rearing is fairly well studied. For example Collins et al. (1975) demonstrated an increase in bacteria in a recirculatory system stocked with channel catfish, *Ictalurus punctatus* (Rafinesque). Jana and Barat (1983) found that fish biomass and the quantity of feed increased heterotrophic bacterial populations in aquaria. Although no food was given to the incubation system during the tests, accumulation of nitrogenous metabolic compounds resulting from continuous egg mortality increased the nutrient levels in the water causing an increase in TVHB numbers.

The increase of TVFS numbers, and specially TVSS numbers, seems to be associated with the ample availability of suitable substrate for fungal propagation as a result of accumulating dead and decaying eggs. Willoughby (1962) reported that unconsumed fish food and dead fish constitute the most important substrate for Saprolegniaceae in fish hatcheries.

Disease-related low egg hatchability and high early fry mortality under hatchery conditions have been reported frequently. However, the mortalities have been attributed to a variety of pathogens, such as *Aeromonas liquefaciens* (Wright and Snow 1975), *A. hydrophilia* (Colesante et al. 1981), *Saprolegnia diclina* (Taylor and Bailey 1979) and *A. prolifera* (Srivastava and Srivastava 1975). This study isolated and identified most of these pathogens as present in the water. Further, scanning electron microscopy revealed the presence of bacteria and fungi on the egg surfaces. However, damage to the chorionic membrane was observed prior to any microbial settlement.

The trend in mouthbrooders is to produce a small number of large eggs which contain large amounts of yolk. Allowed to remain in one position, the yolk accumulates at one pole and disrupts the internal organization, resulting in development failure (Fryer and Iles 1972). In nature, oral incubation is a gentle and delicate process. Further, during the natural incubation process the frequency of "churning" of the eggs by the female within the buccal cavity decreases gradually as development progresses (personal observations). However, in this study, the eggs were kept in continuous circulation. Although the round-bottom incubators provide a somewhat smooth and gentle surface, this agitation could cause mechanical stress on the eggs which could be high. This mechanical damage was evident from the injuries to the chorionic membrane observed within the first 24 hours of incubation. Rana (in press) has already suggested that greater mechanical stress on the egg membrane may result in premature hatching. Therefore, it seems possible that the observed egg mortality was likely to have been associated with mechanical injuries making the eggs susceptible to bacterial invasion.

It is generally believed that the major cause of egg mortality in hatcheries is fungal infections caused by members of the Saprolegniaceae. However, the complete absence of fungal elements on the egg membranes except in association with bacteria, as observed in this study, strongly suggests that fungi, including the identified members of Saprolegniaceae, played only a secondary role in egg mortality.

Artificial incubation systems for mouthbrooding tilapias should be designed to minimize mechanical stress to the eggs, which is very detrimental to their survival. The incorporation of ultraviolet water sterilization units reduces the numbers of potentially pathogenic bacteria. Thus hatchability could be improved, even where a degree of mechanical stress is unavoidable (Subasinghe and Sommerville 1985).

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Table 1. Physicochemical and microbiological qualities of hatchery water (numbers in parentheses are standard deviations).

Parameter	Days from start of experiment 3	24	51
Temperature (°C)	27	27	27
DO (mg/l)	7.2	6.8	7.0
pH	6.8	5.9	7.0
Total NH_3-N (mg/l)	0.08 (0.0)	0.26 (0.03)	0.49 (0.02)
Total NO_2-N (mg/l)	0.001 (0.0)	0.007 (0.002)	0.027 (0.001)
Total viable heterotrophic bacteria cells/ml	1.41×10^3 (0.31×10^3)	1.62×10^4 (0.30×10^4)	4.49×10^4 (0.55×10^4)
Total viable fungal spores/l	11,800 (848)	24,200 (1,838)	35,350 (777)
Total viable saprolegniaceae spores/l	750 (353)	4,875 (176)	9,000 (707)

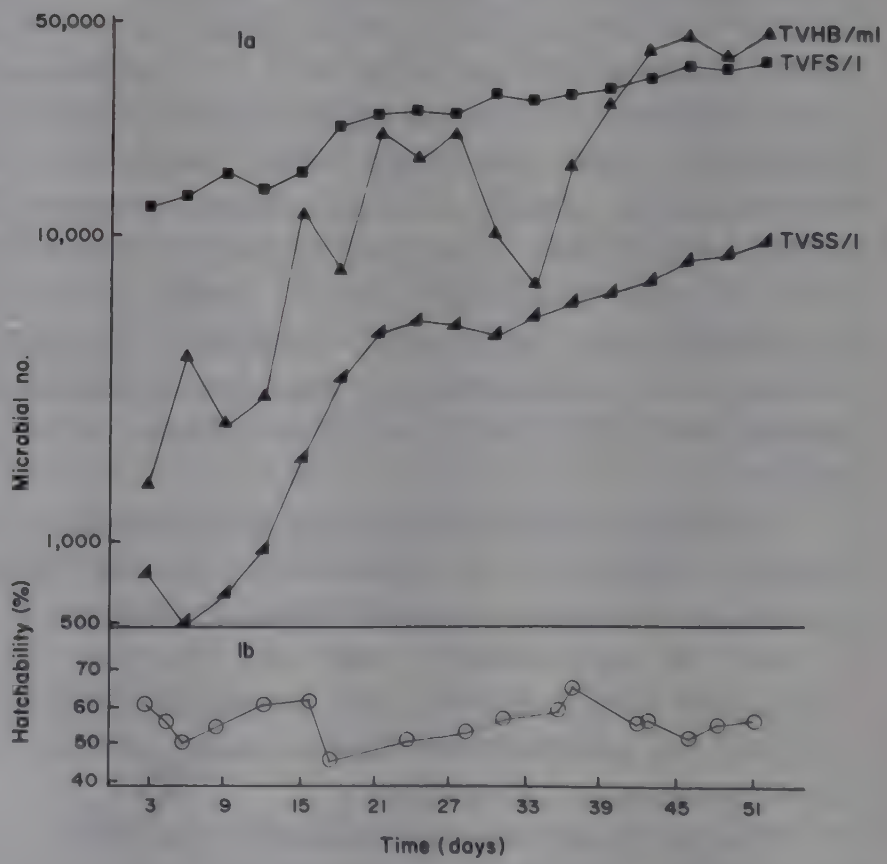

Fig. 1. The pattern of change of the microbiological qualities of the hatchery water and the fluctuation of the hatchability of viable *O. mossambicus* eggs.

Small-Scale Fisheries of Iligan Bay, Philippines

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Abstract

Iligan Bay, one of the important fishing grounds of southern Philippines, has been exploited for years by small- and large-scale fishermen using different types of fishing gears. Speculations on the overfishing of the bay initiated this research project. This study aimed to assess the status and potential of the small-scale fishery of Iligan Bay and was carried out from February to November 1983 in four selected fishing communities along its coastline.

Thirty-three types of small-scale fishing gears operated in the Bay were identified. The most commonly used by small-scale fishermen were handlines *(pasol)*. Designs of this type of gear peculiar to a certain locality were observed and noted. However, they are generally similar. Fish catch data include volume of catch per type of gear per area. Of the fish caught and sampled, 22 families were identified, classified and listed for all fishing gears. Common fishery-related practices of municipal fishermen in the area such as marketing, sharing system and postharvest activities were also noted.

Introduction

Iligan Bay in Northern Mindanao is bounded by three provinces of two regions: Misamis Oriental and Misamis Occidental (Region X) and Lanao del Norte (Region XII). It is one of the most important fishing grounds in southern Philippines and as such has been exploited by fishermen using various types of gears. These fishing gears have been classified by the Philippine Bureau of Fisheries and Aquatic Resources into two categories: the "commercial" and the "municipal". By definition, "municipal" category includes all fishing vessels and craft less than 3 tonnes (t) and all fishing gears that do not require any vessel, are considered small scale. All vessels over 3 t are considered "commercial". In Iligan Bay, only one type of "commercial" gear, locally known as *kubkub* or *likum*, a small type of purse seiner, is used.

Few small-scale fishery-related studies have been conducted in limited areas of Iligan Bay. These are the studies of Herrin et al. (1978) on cooperative and sharing system. In the nearby Panguil Bay areas, fishery household conditions were observed by Hopkins and McCoy (1976).

Suspicion of overfishing the Bay and dissatisfaction among small-scale fishermen over their household conditions motivated this research.

The study aims to document the status of small-scale fishery and the socioeconomic conditions prevailing in the Bay through selected communities. Specifically, the study aims to: (1) assess the status of fish resources; (2) identify small-scale fishing gears, their fishing method, catch and effort and catch economics; (3) examine the marketing and distribution systems; (4) identify income sharing systems; (5) examine household conditions and (6) identify other fishery-related activities.

Materials and Methods

Four fishing communities in Iligan Bay were designated study areas (Fig. 1). These are: Clarin, Cagayanon, Oroquieta, Misamis Occidental and Initao, Misamis Oriental.

Volume of catch per boat per gear was recorded in each study area from February to November 1983. Records of catch for eight days per month were made.

Separate interviews were conducted to determine and identify types of gear used per fishing operation. Information on marketing and distribution of catch was obtained from interviews and actual observations in the local markets and study areas. Household information was collected from 181 respondents, housewives being more responsive to most of the questions.

Results and Discussion

There are 33 types of small-scale fishing gears used in Iligan Bay (Table 1). Almost all types required the use of a boat except for simple handlines and small types of gill nets and drag nets used in shallow waters. Each gear was constructed according to specific size, materials and fishing method applied. Each fishing method is generally intended for catching specific sizes and types of fish. However, some gears were used only during certain seasons.

The most common type of gear used was the simple handline called *pasol*. Fishermen from each selected fishing community (study area) used a peculiar or major type of gear (Table 1). This peculiarity of gear preference might be attributed to the kind of fish resources available in that particular community.

Iligan Bay is composed of several marine aquatic communities. Thus, the Bay offers habitation for various species of fish and invertebrates. As no detailed survey of the fish community was conducted, only fish catches, mostly composed of commercial species, were identified. These fishes could be categorized into four groups: coastal pelagic (anchovy, sardine); oceanic pelagic (tuna, flying fish); hard-reef bottom demersals (reef fishes)and soft bottom demersals (slipmouth).

Stock assessment of the entire fishery resources of Iligan Bay was impossible. From the available data, only a crude estimate could be made of the abundance or availability of some of the important species like anchovy, flying fish and groups of demersal fishes caught with *panti*. Tables 2, 3 and 4 indicate that frequency of gear use determines the total volume of landed catch. However, increase in the number of gears used in the same period would not only mean an increase in total volume of catch, but also a decrease in yield in terms of average catch per gear per trip. Analyses of these data were limited to the catch of gears which was selective in terms of size. However, results indicate that: (1) some resources of the Bay would give profitable yield only to a definite number of gears used for some period of time and (2) some resources are beginning to experience the pressure from the increasing number of fishermen in the area that might lower their yield.

Other significant observations that require serious consideration were those regarding the status of some fishery resources by the fishermen. The majority expressed that there was a considerable decrease in their catch as of 1983 compared to 5 or 10 years ago. They believed that this was due to the increase in the population of their respective communities.

Varied ways of catch-income sharing are practiced in the Iligan Bay area. The common practice is the net-income sharing which stipulates the deduction of expenses for engine fuel, lighting unit and other operating costs from the gross sales and the division of the net profit into three equal shares: one for the boat owner and the two to be divided equally among the fishermen operating the gear. Among the *sari-sari* fishermen, the common practice is a combination of gross-net income sharing (Fig. 2).

The income of the fishermen (engine operator, *arayis* and ordinary crew) and the owner are presented in Table 5. This was calculated from the estimated catch per trip per gear and from the sharing system employed. The income of the owner and owner-operator, however, does not exclude the costs of maintenance which they themselves bear.

The marketing of the fish catch is often managed by the female members of the family, usually by the wife who distributes and markets the fish while the husband maintains the fishing unit. The catch is distributed to, or sold by, several fish vendors *(lab-asera)* through a *suki* system to nearby towns and local markets. *Suki* is a kind of regular seller-buyer relationship between the *lab-asera* and the owner of the fish.

The majority of the fishermen expressed dissatisfaction with their living conditions. They, however, were willing to stay in their communities and continue with fishing as their occupation. They realize that their lack of formal education disqualifies them from other employments (Table 6).

Many of the households had other sources of income (Table 8), but these were temporary and contributed minimally to their total income. These income-generating activities include gathering of marine products (seaweeds, corals, shellfish, etc.) and operating a small retail store.

A young boy of six already accompanied his father in some fishing trips and a fisherman as old as 70 still went fishing.

Household information, gear ownership and fishermen's attitude towards fishing as a means of livelihood are presented in Tables 6, 7 and 8.

Results of this study have significant implications. (1) Increase in the units of major types of gears is likely through added investment. (2) Such increase may not necessarily increase total catch, but may even ultimately reduce profits and subsequently the income of fishermen. (3) Technical improvements in sharing arrangements which determine the distribution of benefits, may probably be difficult. (4) Increased fishing hours and roles of household members in fishing may add only minimal income.

Important problems identified by the research group and also expressed by the majority of the fishermen include: the increasing number of fishermen and gear, the apparent decline of yield of some important commercial fish, low earnings and illegal fishing. These problems can be overcome only if steps are taken to limit the increase of some gears and to formulate and implement effective management schemes of the fishery resources of Iligan Bay.

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Table 1. "Small-scale" fishing gears of Iligan Bay.

Gear type	Visayan name	English name
Line (pasol)	"bundak" or "babit"	multiple hook-jigging method
	"pahawin"	single hook—tuna fishing
	"limbag"	artificial bait troll line
	"aranyas"[1]	ripper
	"lampornas"[2]	multiple hook troll line
	"talonton"	simple hand line (2-5 hooks)
	"pasol"	simple hand line (single hook)
	"pangdiwit"	balance line
	"palagdas"	bottom set long line—crab bait
	"palangre"	bottom set long line
	"palanghoy"	bottom set long line—shark catching
Fish traps	"panggal"	basket type
	"bubu"	large basket type
	"timing"	small basket type
	"bungsod"	fish corral
Bag net and lift net	"basnigan"	round haul seine
	"basnig"	single boat lift net
	"new look"	stationary lift net
Drag net and seine net	"sud-sud"	push net (2 types)
	"baling"	beach seine (2 types)
	"tapsay"	drive-in net—small type
Falling net and gill net	"laya"	cast net
	"panti"[3]	bottom set gill net
	"patuloy"[4]	drift gill net (3 types)
	"sari-sari"[5]	drive-in net—flying fish catching
	"pangsalaaa"	drive-in net—halfbeak catching
	"latak"	encircling gill net

Numeral superscripts denote major types of gears used in selected localities in Misamis Occidental: 1 and 2 Oroquieta; 3 Cagay-anon; 4 Clarin; and 5 Initao.

Table 2. Catch and effort of "patuloy" (daily for 8 days/month).

No. of fish landings/mo.	Total catch (kg)	Catch/trip (kg)
194 (November)	926.5	4.77
173 (April)	1,617.0	9.34
131 (May)	1,457.5	11.12
125 (October)	819.2	6.55
102 (March)	861.0	8.44
99 (February)	1,015.0	10.25
55 (June)	1,039.0	18.89
48 (July)	359.0	7.49
47 (August)	174.0	43.71
47 (September)	475.0	10.10

Table 3. Catch and effort of "sari-sari" (daily for 8 days/month).

No. of fish landings/mo.	Total catch (kg)	Catch/trip (kg)
30 (February)	1,717.2	157.24
16 (October)	3,603.0	214.50
11 (November)	3,680.0	333.54
9 (March)	540.0	60.0
6 (May)	797.0	132.84
3 (April)	29.5	9.80
3 (July)	486.0	156.00
3 (August)	966.0	322.0
1 (June)	255.0	255.0

Table 4. Catch and effort of "panti" (daily for 8 days/month).

No. of fish landings/mo.	Total catch (kg)	Catch/trip (kg)
128 (November)	190.0	1.49
62 (February)	87.3	1.40
57 (March)	100.5	1.76
54 (May)	127.0	2.35
50 (October)	229.1	4.24
31 (June)	88.4	2.85
27 (August)	51.9	1.72
25 (September)	25.4	1.01
10 (July)	29.6	2.06

Table 5. Catch and effort of major gears (February-November 1983).

Gear	Total no. of gears	Trips/month	Total no. of trips	Total catch (kg)		Income/month (₱)
"Sari-sari"	4	12	480	100,308	– Owner "Arayis" – Engine operator – Ordinary crew	6,892
"Patuloy"	21	20	4,200	38,700	– Owner operator	880
"Panti"	7	20	1,400	2,800	– Owner operator	282

Table 6. Personal circumstances and households of fishermen.

Community	No. of household	Mean household size	Average household working member % of fishermen	Average age of head of family	Average education (no. of years)	Average age of fishermen	Households with land (%)
Initao	64	5.48	1.86 / 28.48	38.46	5.24	30.41	7.81
Clarin	29	5.86	2.00 / 70.5	40.55	4.73	32.65	13.79
Cagay-anon	57	5.24	2.22 / 68.46	39.80	6.24	33.34	21.05
Oroquieta	31	4.58	2.06 / 60.67	33.84	5.28	33.84	9.67
Total	181	5.31	2.03 / 69.83	38.62	32.26	5.37	13.08

Table 7. Fishing activity, labor and gear ownership.

Community	Full-time fishermen (%)	Households owning gear (%)	Households owning boats (%)
Initao	90.63	98.87	46.87 / 63.33
Clarin	56.09	100.00	87.09 / 14.81
Cagay-anon	77.01	100.00	82.45 / 57.23
Oroquieta	82.05	93.52	48.38 / 90.00
Total	73.20	97.59	66.10 / 53.64

Table 8. Living conditions and occupational attitudes of fishermen.

Community	% Have to remain in the occupation	% Willing to change location	% Dissatisfied with living conditions	Households with other sources of income (%)
Initao	90.63	42.18	65.63	78.12
Clarin	73.42	20.68	82.76	72.41
Cagay-anon	66.66	21.05	73.69	70.18
Oroquieta	83.88	38.70	69.30	83.87
Average	78.64	30.65	72.84	76.14

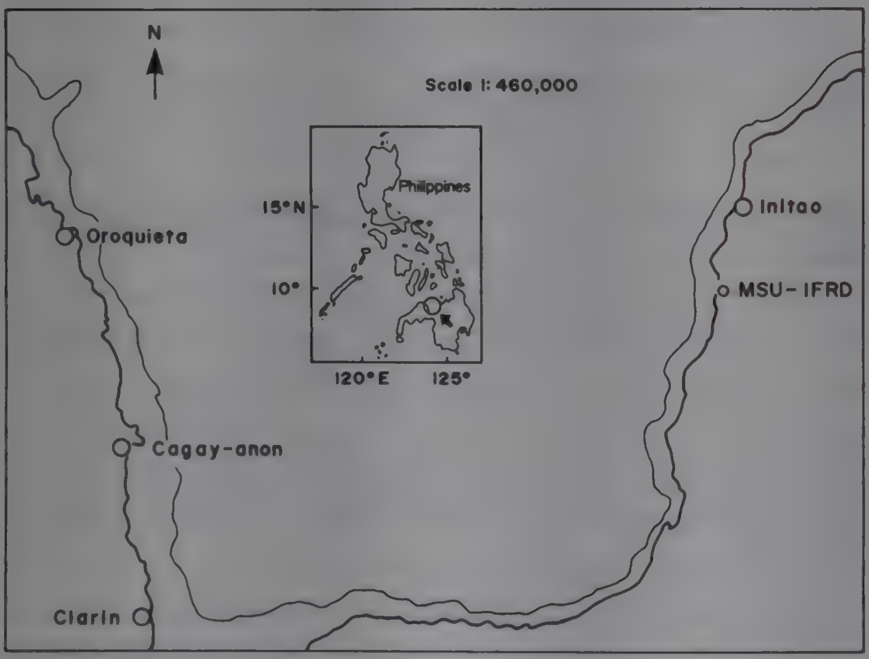
N
Scale 1: 460,000
Philippines
15°N
10°
120°E
125°
Oroquieta
Initao
MSU- IFRD
Cagay-anon
Clarin

Fig. 1. Map of Iligan Bay, Philippines. MSU-IFRD is the site of the Institute of Fisheries Research and Development of Mindanao State University.

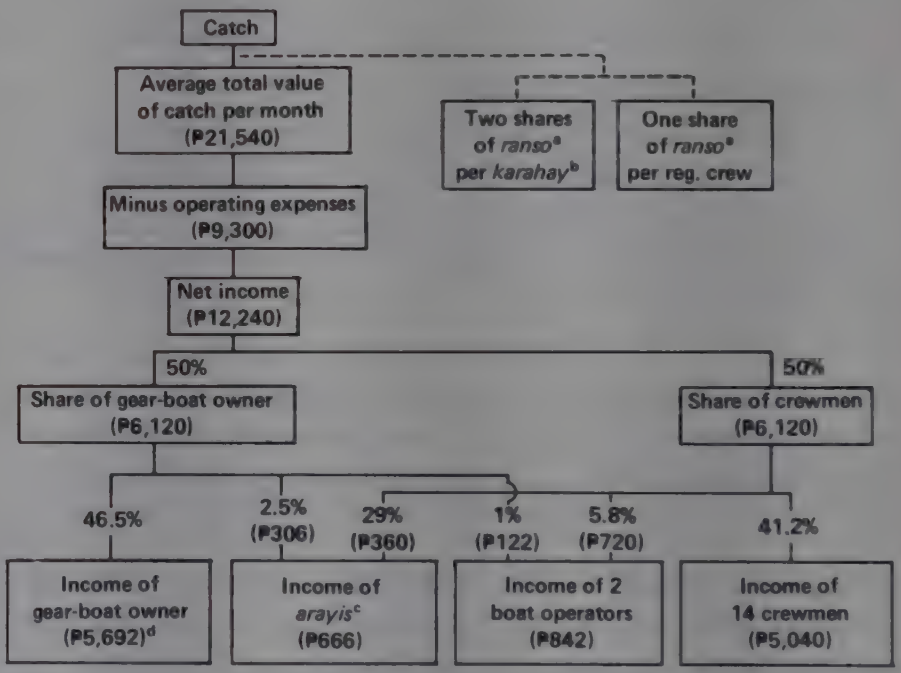
Catch
Average total value of catch per month (₱21,540)
Two shares of ranso^a per karahay^b
One share of ranso^a per reg. crew
Minus operating expenses (₱9,300)
Net income (₱12,240)
50%
Share of gear-boat owner (₱6,120)
50%
Share of crewmen (₱6,120)
46.5%
2.5% (₱306)
29% (₱360)
1% (₱122)
5.8% (₱720)
41.2%
Income of gear-boat owner (₱5,692)^d
Income of arayis^c (₱666)
Income of 2 boat operators (₱842)
Income of 14 crewmen (₱5,040)

Fig. 2. Sharing system of *sari-sari* fishermen of Initao, Misamis Oriental.

[a] Share in kind distributed to crews and *karahay*

[b] Individuals hired for fishing trips but not crew members

[c] Group leader

[d] Before deducting repair and maintenance costs borne by owner

Resource Management is People Management*

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Abstract

In fisheries, traditional emphasis of management programs and research has been on managing the resource rather than managing the people who make use of the resource. This emphasis has led to several misconceptions associated with objectives and mechanisms of fisheries research, training, development and management programs. In this paper, we discuss the nature and character of the fisheries management problem. We argue the need for, and present the basic requirements of, an integrated approach to fisheries management.

Introduction

In fisheries, especially small-scale fisheries, the traditional emphasis and concern of management has been on the resource component rather than on the social component consisting of the individuals and institutions which participate in the use of the resource (Emmerson 1980).

Scientific knowledge has developed with this emphasis on "getting to know the fish" their distribution, growth and mortality rates, food and migratory habits, taxonomy, morphology, physiology, pathology, genetics, etc.

Much slower has been the development of scientific knowledge that would help explain the motivations and behavior of fishermen and fish consumers and the socioeconomic organizations which serve to "institutionalize" their actions, and the many, often complex, interrelationships between the main actors in the fishery. Most seriously, perhaps, little is known about how the various actors react to various fisheries management policies.

Thus, fisheries managers who are responsible for seeing that the highest sustained benefits from the exploitation of the fishery flow to society, face a difficult and frustrating task. In terms of managing the fishery, what they know most about (the fish and the catching technologies) is the least useful information, and what they know least about (the socioeconomic relationships within the fishery) is the most useful information.

In this paper, we discuss the nature and character of the fisheries management problem. We argue the need for, and present the basic requirements of, an integrated approach to bridging this information gap.

Nature and Character of Fisheries Management

Natural resources provide a wide variety of benefits to society, from purely aesthetic values to the provision of physical inputs that satisfy our material needs. Some resources, such as minerals, are exhaustible (depletable) while others, such as fisheries and forests, are renewable (replenishable). Living aquatic resources are renewable in the sense that, within limits and after due lapse of time, the biomass has the natural capacity to regenerate itself towards its original environmentally stable equilibrium level after disruption due, for example, to harvesting or pollution. A fishery can provide a certain yield in perpetuity if fish removal does not exceed natural reproduction levels. Often the conditions under which yields can be maintained over time have been misunderstood in the sense that the resource has been regarded as so vast that no amount of exploitation could tip the balance between its natural ability to rejuvenate itself and its equally natural ability to be driven into extinction.

But we know that in many unmanaged or improperly managed fisheries, particularly where population growth and/or economic development have increased the demand for fish, where there is free access to the resource, and where fishermen have responded to these conditions with new technologies and larger fleets, the results have not been more and cheaper fish in the markets, but economic overcapitalization, biological overexploitation of the resource, and a generally depressed and declining fishing industry.

When faced with this kind of problem the usual management approach has been to attempt to identify the maximum sustainable yield (MSY) and its associated parameters for selected species for the resource and, through various measures, to see that this limit is not exceeded. Moreover, by assuming that all harvested fish have some positive benefit for society, management attempts to achieve harvests at the MSY level with increased technical efficiency and least costs. The commonly applied mechanisms are laws, regulations and enforcement measures which deal exclusively with fishing effort and resource access systems based on purely biotechnical considerations (Smith 1979). We maintain that the biotechnical approach treats the symptoms of the diseased fishery rather than the disease itself.

From the resource management point of view, overfishing, overcapitalization, excessive effort and rent dissipation are undesirable results of the resource allocation mechanism. They are signals of a failure in the socioeconomic and institutional arrangements designed by society (the community) for resource use purposes: the resource property system (laws, regulations) with its associated access system, the incentives (social, economic, psychological) for resource exploitation, and the stable links (structure) established among the participants of the resource exploitation activity.

Individuals, communities and the society define the value (benefit) of a resource and generally, also the measure of relative scarcity of the resource (Leuschner 1984). The management problem arises when the resource becomes scarce, not scarce in absolute terms but scarce relative to society's use or demand for the resource. In developed countries the valuation of resources is generally a function of the market or of a central planning authority. In developing countries, the market or central authority is important also, but other factors such as tradition, religion, habits and beliefs may carry considerable weight (Ruddle and Johannes 1985). International trade contributes to the value equalization process through relative prices of tradeable goods but various externalities, trade unions, tariff and nontariff barriers to trade, exchange rate differentials and market atomization make national, regional and local social and institutional factors fundamental in assessing the value of resources.

Thus "value" and "scarcity" are closely related concepts and trying to manage a scarce resource without consideration of its local, sectoral and spatial value is a futile excercise. This is especially important in the management of small-scale fisheries where market isolation, factor immobility, nonprofit maximizing decisions, nonmarket transactions and many other social, cultural, economic, institutional and political considerations prevent simple extrapolations from the purely biotechnical rationale of fishery management (Panayotou 1982). Some of the key factors which must be well understood before attempting to manage fishery resources are the relative values in predator-prey and fish-environment relationships, the alternative uses of water for fisheries, agriculture, industry and others, the intricate market-taste-income determinants of relative scarcity, and the relative value of productive factors involved in fisheries. This is information about the people who use the resource, about the institutions people create for managing their resources, and about the links, rules, procedures and behavioral reactions of people in the procurement of goods.

The Need for an Integrated Approach to Research and Management

Management of a fishery usually involves interfering in the fishery process with the object of changing the process towards the most desired social benefit from the resource. In doing this managers make use of contemporary social tool called "laws and regulations" which influence people's behavior in particular matters. Thus, an essential component of fishery management is people, and a necessary component for rational and effective management is the accurate consideration of people's reaction to the management program itself.

Since the pioneering work of Gordon (1954), extensive and rigorous theoretical developments have taken place, aided by sophisticated mathematical models, which give more emphasis to bio- and technoeconomic relationships and allow practitioners to incorporate stochastic, dynamic and uncertainty elements into their analytical frameworks (Clark 1985) both at the micro and macro levels. Unfortunately, most of this scientific knowledge remains at the academic level and the empirical base for the models and their conclusions is still weak. Because of this, fishermen and fisheries managers are reluctant to make use of these findings and, although some models have been converted into simple, fast and efficient interactive computer programs and highly consistent mathematical models, they fear that by using them they might get involved in unrealistic and useless exercises.

The gap between theoretical findings and practical applications remains wide and management decisions continue to be based on limited and partial information which usually has a strong bias towards efficiency (technical) and biological (conservation) considerations. This situation implies that more research and information must be generated on the socioeconomic and institutional factors which govern the operation of a fishery. The need is not only for more information but for a different kind of research and knowledge. The fishery process consists of interrelationships between many different elements and while each science (biology, physics, genetics, sociology, economics) specializes in developing certain kinds of knowledge about some elements, none is able to explain the fishery system or process as a whole.

The present need is for a set of testable propositions about the way each relevant element or component of the fishery is functionally related to the rest. We can expect that these relationships will be quite complex to account for the stochastic, dynamic and multivariate nature of the relevant factors. The theoretical background whence these propositions should come certainly requires interdisciplinary effort to comprehend the system as a whole. This alone is not capable of providing the needed knowledge. The synthesis will come only as the propositions are applied, tested and monitored in the field.

Requirements for Effective Fisheries Management

In the discussion so far we have shown some basic requirements for the avoidance of fisheries management failures. Three sets of requirements need emphasis.

First is the need for feasible management programs. The feasibility of management programs is generally tested on financial and technical grounds and decisions are usually based on traditional indicators of profitability such as internal rate of return, cost-benefit ratios and break-even point analysis (Campleman 1976). Unfortunately, these micro measures are often generalized to macro (industry or sector) levels without consideration of the dynamic elements of the fishery process. Project evaluation techniques must be developed to incorporate, in addition to "social" values, such dynamic variables as cost per unit effort (cost structure) and catch per unit effort (population dynamics) at different levels of effort in relation to market prices (income). Potential conflicts between industry and small-scale fishermen, market interactions that could adversely affect small-scale fishermen, and sociocultural and institutional impacts of technological changes should also be built into the evaluation of fisheries projects.

Second is the necessity to accept the need for management in a fishery. Because scarcity and management involve problems of temporal and spatial aggregation and allocation, it is sometimes difficult to persuade fishermen and some professionals in management positions to accept that there is a need for management policies to improve the use of a resource which appears to be showing positive yields. The fundamentals of the biotechnoeconomic dynamic relationships and the sociocultural conditions preventing the attainment of the greatest possible benefit to society from the resource must be well understood and accepted by those who make the management decisions and those who are affected by them. Without a widespread understanding of the basic issues and reasons for a particular management action, ways and means will be devised to avoid compliance and/or to weaken enforcement. This is where training and extension can play an important part in fishery management.

Finally, there is the need for a database and information system for management purposes. The present databases and information systems of most countries are inadequate for effective fisheries management purposes. Their weaknesses and deficiencies have been well documented and efforts are being made by leading institutions to correct this most fundamental and serious management problem.

Considerable financial and technical resources would be required to design, set up and operate an information system that could adequately meet the needs of fishery management and research. While the purpose would be to collect and organize data clearly, rather than to presuppose particular cause-and-effect relationships *a priori*, the system also must have the capability for conceptualizing, planning and forecasting. Abundant literature now exists on database concepts, systems and construction and the recent worldwide expansion of the microcomputer industry offers magnificent (and cost effective) possibilities that were unthinkable a few years ago. While the costs of setting up an adequate management information system could be quite high, these costs should be weighed against the potentially substantial benefits of improved fisheries management.

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Characteristics of an Exploited Tropical Shallow-Water Demersal Fish Community in Malaysia

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Abstract

Trawling provided insights into the characteristics of an exploited tropical shallow-water demersal fish community. A total of 6,565 fish specimens weighing 285 kg were caught at 20 sampling stations. In all, 139 species belonging to 50 families were recorded. The major families ranked by weight were Dasyatidae (19.7%), Synodontidae (18.3%), Paralichthyidae (8.9%), Dactylopteridae (8%), Nemipteridae (5.3%), Lagocephalidae (5.2%), Priacanthidae (5%); and Mullidae (4%). The overall fish trawled consisted of 53% food fish and 47% trash fish. The demersal fish community could be partitioned into four trophic groups, i.e., large zoobenthos feeders, intermediate predators, small demersal zoobenthos feeders and small demersal zooplankton feeders. Small crustaceans played an important role as food resources for all the trophic groups. They were the major food for small demersal zoobenthos feeders, the dominant group, and large zoobenthos feeders. Analysis of growth characteristics of ten common species using length-frequency data showed that *Saurida elongata* and *Trachinocephalus myops* (Synodontidae) and *Dactyloptena orientalis* (Dactylopteridae) had higher growth rates than the other fishes in the community. Exploitation rates of these three species by trawlers were also high although they have little commercial value. Annual recruitment patterns for the demersal fishes were generally protracted showing a single pulse, although some species have a second minor pulse.

Introduction

The management of tropical multispecies fisheries has long been seen as a challenge to fishery scientists. The constraints are numerous and well-defined (Pauly 1979; Marr 1982). Among some of the obvious constraints are lack of theory and databases. Larkin (1982) outlined the requirements for research ranging from basic data collection to development of theory applicable to multispecies tropical fisheries.

This study was designed to provide insights into the characteristics of an exploited demersal fish community. It had three major objectives: a) to provide information on the composition of the fish stocks; b) to determine the food habits among the demersal fishes; and c) to characterize the growth rates, exploitation rates and annual recruitment patterns for the common species.

Materials and Methods

Data were obtained through a trawling program conducted within 12 km off the coastline of Terengganu, Malaysia, where the depth of the water did not exceed 20 m (Fig. 1). This area is suitable for trawling with respect to bottom characteristics (medium coarse to fine sand with some muddy patches) and is intensively exploited by small commercial trawlers and purse seiners below 40 gross tons and by artisanal fishermen.

Trawling was conducted using an otter trawl net with an effective wing-span of 6 m and a cod-end mesh size of 38.1 mm. Trawling was maintained for approximately 60 min. at a tow speed of 2.3-2.4 knots. A total of 20 sampling stations were covered in six trawl operations conducted between July 1984 and April 1985 (Table 1 and Fig. 1). Trawling was suspended during the northeast monsoon from November to March.

Each haul was sorted to species and weighed. Samples for stomach content analysis were immediately preserved in 8% buffered formalin. Qualitative analysis of the stomach contents for the species caught enabled them to be partitioned into general feeding groups. Representative species from each group were also taken for quantitative stomach content analysis using the gravimetric method. Food groups in the stomachs were categorized as 1, intermediate predators; 2, pelagic fish; 3, small demersal zoobenthos feeders; 4, small demersal zooplankton feeders; 5, heterotrophic benthos (octopus, cuttlefish and echinoderms); 6, large crustaceans; 7, small crustaceans; 8, small molluscs and worms; 9, meiobenthos; and 10, zooplankton.

Length-frequency data were also recorded for ten common species. ELEFAN I and ELEFAN II programs described by Ingles and Pauly (1984) were used to estimate growth parameters (L_∞ and K), mortality (total mortality, Z; fishing mortality, F; and natural mortality, M), exploitation rates (E), length at first capture (L_C) and annual recruitment patterns.

Results and Discussion

The trawl data are shown in Table 1. In all, 6,565 specimens weighing 285 kg were obtained. The overall percentage contribution of food fish (53% by weight) was comparable to that of trash fish (47% by weight). These figures, although vastly different from values in one report (Anon. 1967), do not differ much from figures given by Pathansali et al. (1974), Jothy et al. (1975) and Lam et al. (1975) from research trawl surveys conducted in waters 10-20 m deep off the coast of Terengganu. However, the percentage contribution of food fish may be grossly overestimated since many of the food species caught were composed of small fish.

Of the 139 species belonging to 50 families recorded, 75 were food fishes. The most abundant species by weight were *Dasyatis zugei* (15.4%), *Saurida elongata* (10.2%), *Dactyloptena orientalis* (8%), *Trachinocephalus myops* (7.8%), *Pseudorhombus javanicus* (5.9%) and *Priacanthus tayenus* (5%) (Table 2). The families are listed by weight in Table 3.

In a virgin stock before the introduction of trawling, Leiognathidae and rays (Dasyatidae included) were the two most abundant food fish families followed by Tachysuridae, Carangidae, Nemipteridae and Pomadasyidae (Anon. 1967). Data from this study, which reflect the effects of 18 years of trawling, show certain deviations from the composition of a virgin stock. While Dasyatidae still dominates in biomass, Leiognathidae had decreased significantly, presently contributing only 2.78% of the total biomass, compared to 12.79% in a virgin stock. Pauly (1979) similarly found sharp declines in the abundance of Leiognathidae as a result of trawling. Trash fish families, Synodontidae, Paralichthyidae, Dactylopteridae and Lagocephalidae seem to feature significantly in an intensively exploited stock while important food fish groups include Nemipteridae, Priacanthidae, Mullidae and Carangidae.

Qualitative stomach content analysis showed that the demersal species could be conveniently grouped into four feeding levels. These were large zoobenthos feeders (6 species), intermediate predators (29 species), small demersal zoobenthos feeders (81 species) and small demersal zooplankton feeders (8 species).

Food composition data for large zoobenthos feeders were obtained for three species, i.e., *Dasyatis uarnak, D. zugei* and *Drepane punctata* (Table 4). Small crustaceans featured as the major food group, making 66.4% of the diet by weight, followed by large crustaceans (26.3%), small molluscs and worms (7%) and heterotrophic benthos (0.3%).

The food groups of intermediate predators are shown in Table 5. Small demersal zoobenthos feeders constituted the most important food group (76.5%) followed by small demersal zooplankton feeders (8.4%), large crustaceans (7.7%) and small crustaceans (4.3%). Pelagic fish, intermediate predators and heterotrophic benthos contributed only minimally to the food items.

The most important food group for small demersal zoobenthos feeders was small crustaceans which contributed about 53.6% of the diet, followed by heterotrophic benthos (15.4%), small molluscs and worms (11.5%), large crustaceans (9.9%) and small demersal zooplankton feeders (5.7%). Small pelagic fish and small demersal zoobenthos feeders were found only in the stomachs of *Priacanthus tayenus* and *Nemipterus* spp. and played a minor role as a food group (Table 6).

Only three species of small demersal zooplankton feeders were included for stomach content analysis (Table 7). These fed only on a small range of food groups, i.e., small crustaceans, small molluscs and worms and zooplankton. *Daya jerdoni* and *Centriscus scuttatus* fed exclusively on zooplankton while *Pentaprion longimana* fed on small crustaceans and small molluscs and worms as well, but retaining zooplankton as the major food group. The overall food composition ratios are shown in Table 7.

Many species showed trophic similarities suggesting a certain degree of competition on the same food groups. Competition for the same food groups occurred within the same feeding level as well as between different feeding levels. Small crustaceans seem to play a major role as a food resource and were found in the stomachs of most of the demersal fish present. They also constituted the major food group for small demersal zoobenthos feeders and large zoobenthos feeders which made up 95% of the total biomass of the demersal fish present. The most abundant feeding group, i.e., small demersal zoobenthos feeders contributed largely to the food of intermediate predators and would account for the relative abundance of the latter (45% of the total biomass). Hacunda (1981) similarly found crustaceans to be the major prey group in all demersal predators present in a coastal area of the Gulf of Maine and concluded that predators rely on the same major food sources. He also provided data to show that trophic partitioning by prey size occurred and this would help reduce intense competition on similar food groups. Differences in daily, seasonal and spatial patterns of feeding could provide another means of reducing interspecific competition. Keast (1973) found that food overlap occurs when a particular food resource becomes superabundant. This could be the case for small crustaceans in the shallow-water habitats off Terengganu.

A summary of the growth parameters, mortality rates, exploitation rates and annual recruitment patterns of 10 common species caught are given in Table 8. Higher growth rates, as indicated by the growth coefficient, k, were observed in *Saurida elongata, Trachinocephalus myops, Dactyloptena orientalis* and *Gastrophysus* *scleratus* than in *Priacanthus tayenus, Leiognathus elongatus* and *Daya jerdoni.* Exploitation rates were also high for *Saurida elongata, Trachnicephalus myops* and *Dactyloptena orientalis* although these fishes have little commercial value. Annual recruitment patterns for the demersal fishes analyzed are shown in Fig. 2. Recruitment patterns were generally protracted, showing a single pulse as in *Upeneus sulphureus, Gastrophysus scleratus, Saurida elongata, Trachinocephalus myops* and *Pseudorhombus javanicus.* However, *Pentaprion longimana* had a second pulse, similar to that reported by Ingles and Pauly (1984) for this species in the Philippines.

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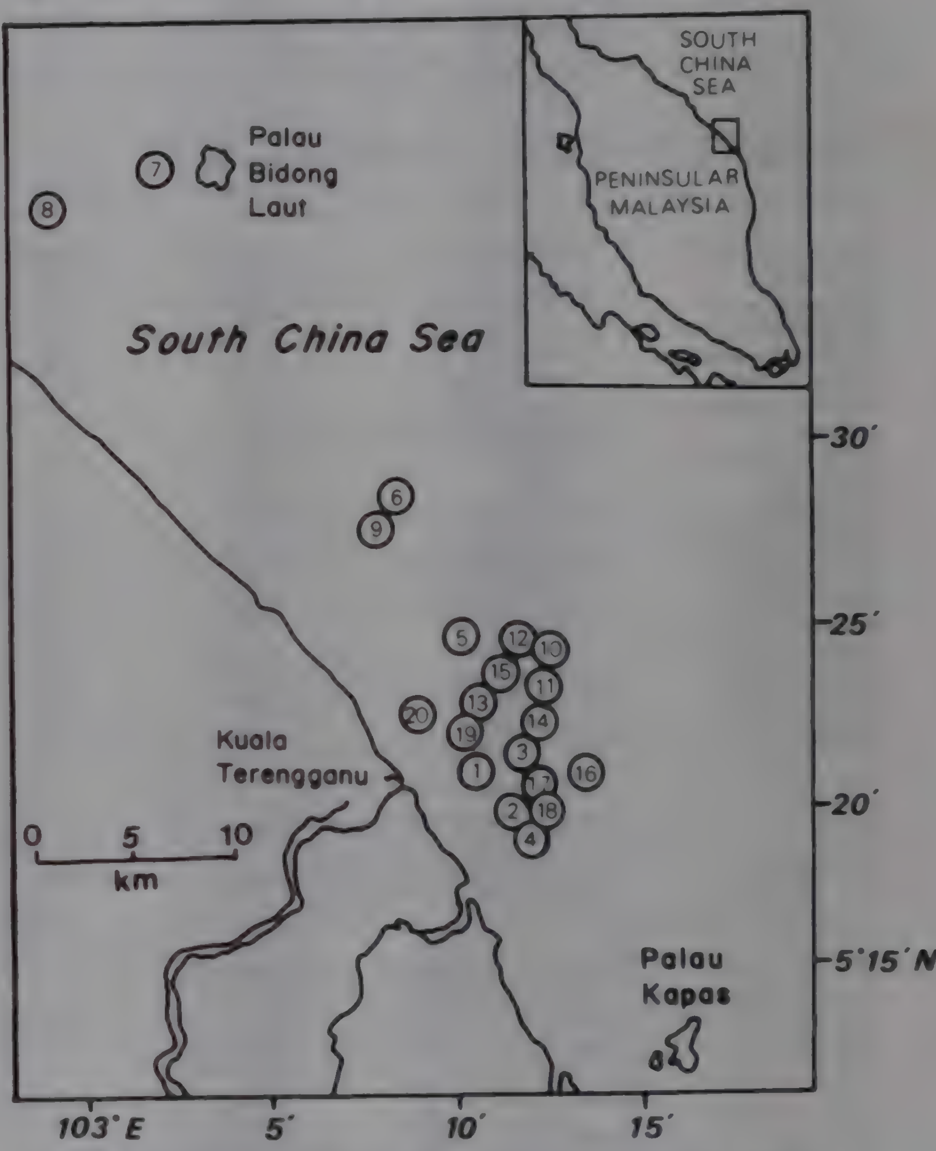

Fig. 1. Study area showing the location of sampling stations.

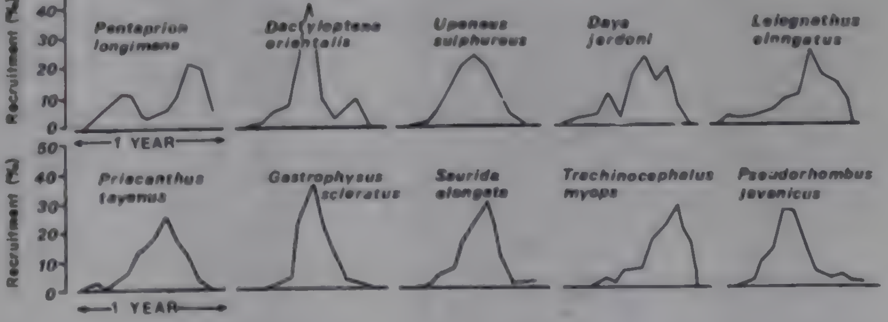

Fig. 2. Annual recruitment patterns for common demersal fish.

Table 1. Summary of trawl data, Terengganu, Malaysia, July 1984-April 1985

Station	Sampling data	Total wt of fish (g)	Total no. of fish	Food fish % by no.	Food fish % by wt.	Trash fish % by no.	Trash fish % by wt.
01	22.07.84	2,175	103	77	43	23	57
02	22.07.84	28,135	830	43	49	57	51
03	16.08.84	36,904	841	67	66	33	34
04	16.08.84	22,335	606	63	45	37	55
05	23.08.84	23,831	393	44	56	56	44
06	23.08.84	6,900	388	5	3	[illegible]	97
07	23.08.84	16,138	35	20	82	80	18
08	23.08.84	1,870	129	30	54	70	46
09	17.09.84	6,109	124	1	1	99	99
10	17.09.84	34,135	831	18	15	82	85
11	17.09.84	15,739	405	24	33	76	67
12	17.09.84	12,090	284	26	15	74	85
13	22.10.84	9,587	346	64	51	36	49
14	22.10.84	1,810	52	83	79	17	21
15	07.04.85	19,453	58	47	92	53	8
16	07.04.85	2,226	122	14	20	86	80
17	07.04.85	14,707	108	56	92	44	8
18	07.04.85	4,618	251	52	63	48	37
19	07.04.85	8,786	225	52	76	48	26
20	07.04.85	17,749	435	69	78	31	22
Total		284,897	6,565	mean = 44	mean = 53	mean = 56	mean = 47

Table 2. Major species caught by demersal trawls listed in descending order by weight.

Species	Family	Weight (g)	% by wt.
Dasyatis zugei	Dasyatidae	43,900	15.41
Saurida elongata	Synodontidae	28,942	10.16
Dactyloptena orientalis	Dactylopteridae	22,890	8.03
Trachinocephalus myops	Synodontidae	22,343	7.84
Pseudorhombus javanicus	Paralichthyidae	16,820	5.90
Priacanthus tayenus	Priacanthidae	14,358	5.04
Gastrophysus scleratus	Lagocephalidae	9,724	3.41
Dasyatis sp.	Dasyatidae	9,000	3.16
Upeneus sulphureus	Mullidae	6,887	2.42
Arius thalassinus	Ariidae	4,970	1.75
Daya jerdoni	Pomacentridae	4,622	1.62
Carangoides malabaricus	Carangidae	4,279	1.50
Leiognathus elongatus	Leiognathidae	4,038	1.42
Pseudorhombus sp.	Paralichthyidae	4,038	1.42
Scolopsis taeniopterus	Nemipteridae	3,800	1.33
Gastrophysus spadiceus	Lagocephalidae	3,600	1.26
Drepane punctata	Drepanidae	3,315	1.16
Gymnocranius griseus	Pentapodidae	3,135	1.10
Sphyraena sp.	Sphyraenidae	3,100	1.09
Dasyatis uarnak	Dasyatidae	2,910	1.02

Table 3. Fish families in the demersal trawls listed according to weight.

Family	Weight (g)	% by weight
Dasyatidae	56,170	19.715
Synodontidae	52,039	18.265
Paralichthyidae	25,438	8.928
Dactylopteridae	22,890	8.034
Nemipteridae	15,038	5.278
Lagocephalidae	14,734	5.171
Priacanthidae	14,368	5.043
Mullidae	11,718	4.113
Carangidae	7,951	2.790
Leiognathidae	7,918	2.779
Ariidae	5,470	1.919
Pomacentridae	5,082	1.783
Pentapodidae	4,650	1.632
Lutjanidae	3,594	1.261
Sphyraenidae	3,534	1.240
Drepanidae	3,315	1.163
Platycephalidae	3,230	1.133
Apogonidae	3,187	1.118
Pomadasyidae	2,941	1.032
Gerridae	2,682	0.941
Bothidae	2,522	0.885
Siganidae	2,201	0.772
Psettodidae	2,026	0.711
Orectolobidae	1,800	0.631
Callionymidae	1,547	0.543
Monocanthidae	1,076	0.377
Triacanthidae	1,070	0.376
Sciaenidae	743	0.260
Lethrinidae	728	0.255
Balistidae	624	0.219
Soleidae	569	0.199
Sillaginidae	568	0.199
Cynoglossidae	540	0.189
Ostraciidae	425	0.149
Coridae	350	0.122
Diodontidae	346	0.121
Centriscidae	325	0.114
Tetraodontidae	320	0.112
Serranidae	256	0.089
Theraponidae	248	0.087
Fistulariidae	196	0.068
Torpedinidae	115	0.040
Ephippidae	109	0.038
Scorpaenidae	50	0.017
Synancejidae	50	0.017
Uranoscopeidae	50	0.017
Gobiidae	45	0.016
Formionidae	26	0.009
Pegasidae	15	0.005
Triglidae	10	0.003
Total weight	284,897	

Table 4. Food composition ratios for large zoobenthos feeders. See text for food group designations.

Large zoobenthos feeders	Food groups 5	6	7	8	Weight (kg) caught	Relative fraction
Dasyatis uarnak	0	0	.88	.12	2,910	.058
Dasyatis zugei	0	.30	.70	0	43,900	.876
Drepane punctata	.05	0	0	.95	3,315	.065
Overall ratio	.003	.263	.664	.070		

Table 5. Food consumption ratios for intermediate predators. See text for food group designations.

Intermediate predators	Food groups 1	2	3	4	5	6	7	Weight (kg) caught	Relative fraction
Saurida elongata	0	0	.646	.120	0	.234	0	25.33	.304
Pseudorhombus spp.	0	0	.800	.088	0	0	.112	23.11	.277
Trachinocephalus myops	0	0	.989	.011	0	0	0	21.47	.258
Arius thalassinus	0	0	.700	0	0	.100	.200	4.97	.060
Sphyraena spp.	.100	.500	.200	.200	0	0	0	3.53	.042
Lutjanus spp.	0	0	.726	.089	.179	.006	0	2.97	.036
Psettodes erumei	0	0	.700	.300	0	0	0	1.41	.017
Saurida undosquamis	0	0	.850	.120	0	.030	0	.35	.004
Fistularia spp.	0	0	0	1.000	0	0	0	.16	.002
Overall ratio	.004	.021	.765	.084	.008	.077	.043		

Table 6. Food consumption ratios for small demersal zoobenthos feeders. See text for food group designations.

Small demersal zoobenthos feeders	Food groups 2	3	4	5	6	7	8	9	10	Weight (kg) caught	Relative fraction
Dactyloptena orientalis	0	0	0	0	.108	.892	0	0	0	18.59	.250
Priacanthus tayenus	.139	0	.091	0	.012	.728	.030	0	0	7.20	.097
Upeneus spp.	0	0	0	0	.143	.857	0	0	0	10.23	.138
Nemipterus spp.	0	.101	.215	.295	.366	.023	0	0	0	8.87	.120
Gastrophysus spp.	0	0	0	.914	0	.086	0	0	0	9.46	.127
Leiognathus spp.	0	0	0	0	0	.400	.600	0	0	5.45	.073
Gymnocranius griseus	0	0	0	0	0	0	1.000	0	0	2.88	.039
Scolopsis taeniopterus	0	0	.026	0	.206	.510	.258	0	0	2.62	.035
Engyprosopon spp.	0	0	0	0	0	.800	.200	0	0	2.16	.029
Apogon spp.	0	0	0	0	0	.800	0	0	.200	3.05	.041
Siganus spp.	0	0	0	.100	0	.200	.500	.200	0	1.89	.026
Gazza minuta	0	0	.843	0	0	.102	.055	0	0	1.83	.025
Overall ratio	.014	.012	.057	.154	.099	.536	.115	.006	.008		

Table 7. Food consumption ratios for small demersal zooplankton feeders. See text for food group designations.

Small demersal zooplankton feeders	Food groups 7	8	10	Weight (kg) caught	Relative fraction
Pentaprion longimana	.18	.20	.62	1.61	.308
Daya jerdoni	0	0	1.00	3.29	.629
Centriscus scuttatus	0	0	1.00	.33	.063
Overall ratio	.055	.062	.883		

Table 8. Growth, mortality and exploitation parameters for 10 common demersal species.

Species	L_∞ (cm)	K	Z	M	F	E	Lc (cm)
Pentaprion longimana	16.0	1.1	3.8	2.3	1.5	0.4	8.7
Dactyloptena orientalis	23.0	1.5	10.6	2.5	8.1	0.8	12.5
Upeneus sulphureus	23.0	1.1	5.0	2.1	2.3	0.6	11.0
Daya jerdoni	17.0	0.8	3.1	1.8	1.3	0.4	5.6
Leiognathus elongatus	13.5	0.8	2.4	1.9	0.5	0.2	8.2
Priacanthus tayenus	27.0	0.6	2.9	1.3	1.6	0.6	13.0
Gastrophysus scleratus	18.0	1.5	4.2	2.7	1.5	0.4	10.9
Saurida elongata	37.0	1.6	7.5	2.3	5.2	0.7	21.9
Trachinocephalus myops	36.5	1.6	8.0	2.3	5.7	0.7	17.4
Pseudorhombus javanicus	26.5	1.2	2.4	2.1	0.3	0.1	21.1

Trends in Reservoir Fisheries in Sri Lanka with Special Reference to *Etroplus suratensis, Tilapia rendalli* and *Oreochromis niloticus*

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Abstract

The inland fishery of Sri Lanka is essentially a fishery for the exotic *Oreochromis mossambicus* in reservoirs. Recent changes observed in the fishery of five reservoirs in relation to indigenous *(Etroplus suratensis)* and other exotic cichlids *(Tilapia rendalli* and *Oreochromis niloticus)*, introduced to Sri Lanka in 1969 and 1975, respectively, are described. In the reservoirs of Udawalawe and Mahagama, *E. suratensis* catches registered an increase from 0.72 to 12.0% and from 0.46% to 28.36%, respectively, during the last ten years. *E. suratensis* and *T. rendalli* together contribute 53.6% to the fishery in Mahagama, whereas their contributions to the fishery in Udawalawe, Chandrikawewa and Kiriibbanarawewa are 19.5%, 18.6% and 21.0% respectively. The abundance of macrophytes, particularly *Ipomea aquatica*, in Mahagama reservoir may be seen as the cause for the overwhelming dominance of *E. suratensis* and *T. rendalli*, which are known to be efficient macrophyte feeders. In Soraborawewa, drastic reduction of *O. mossambicus* in the fishery was observed and *O. niloticus* became the dominant species four years after it was introduced. In this reservoir, the abundance of blue-green algae not utilized by *O. mossambicus* appears to have favored *O. niloticus*. The abundance of blue-green algae in the phytoplankton of other reservoirs in Sri Lanka suggests the desirability of judicious stocking of *O. niloticus* for increased production from these waters.

Introduction

The inland fishery of Sri Lanka is essentially totally confined to the manmade lakes, and much has been written on these types of reservoirs, their relevance to the inland fishery and strategies for their development (Fernando and Indrasena 1969; Fernando 1977; Fernando and De Silva 1984). It is still artisanal with marginal scientific management. More recently, De Silva (1985), taking into consideration twenty major reservoirs, suggested a management strategy to optimize fish yield. All the earlier studies were on *Oreochromis mossambicus*, the dominant reservoir species. This paper describes changes in the fishery of five major reservoirs in relation to three macrophyte-feeding cichlids, the Asian cichlid, *Etroplus suratensis* (Bloch) and the exotic *Tilapia rendalli* and *Oreochromis niloticus* (Linne).

Materials and Methods

Fish catch statistics and published data have been utilized to show the changes in species composition of the five reservoir fisheries in Sri Lanka, the location and details of which are in Table 1, as reported earlier (Maitipe and De Silva 1984; De Silva 1985). These reservoirs were selected because of the availability of reliable and relatively long-term data on their fishery.

Results and Discussion

Changes in the species composition of the catches of two southern reservoirs, Mahagama and Udawalawe, in 1973-1984, to the extent available, are given in Fig. 1 (a and b), and those of Chandrikawewa and Kiriibbanarawewa in 1974 and 1981-1984 in Table 2.

Catches of *E. suratensis* from the Udawalawe reservoir have gradually increased from 0.72% (1973) to 11.95% (1982) and from the Mahagama reservoir, from 0.46% in 1973 to 28.36% in 1982. Species composition of fish catch of Chandrikawewa in 1981-1984, however, does not show appreciable increase in contribution of *E. suratensis* from the 1974 level which was 5.2% (Fernando 1977). In Kiriibbanarawewa *E. suratensis* contributed 7.6% in 1981-1984.

The contributions of the exotic cichlid, *T. rendalli* (introduced in 1969 from East Africa) to the fishery of Udawalawe, Chandrikawewa and Kiriibbanarawewa appear to be similar to that of *E. suratensis*. In Mahagama reservoir the contribution of *T. rendalli* to the fishery in 1981-1984 was 36.34% (range 23.2-57.9%), significantly higher than in Udawalawe (10.0%), Chandrikawewa (10.5%) and Kiriibbanarawewa (13.4%). In Mahagama *E. suratensis* and *T. rendalli* contributed 53.24%, and 19.48%, 18.12% and 21.0%, respectively, in Udawalawe, Chandrikawewa and Kiriibbanarawewa. The relative contribution of *T. rendalli* in 1980 in the Udawalawe (27.8%) and Mahagama (57.9%) reservoir was significantly higher than in other years.

T. rendalli is a macrophyte feeder. Macrophyte-feeding cichlids have been reported to contribute significantly to reservoir fisheries in years immediately following their introduction, as in the case of *T. zillii* in Teso Dams, Uganda (Lowe-McConnell 1982). *T. zillii* continued to dominate the Teso Dams fishery until the vegetation thinned out and *S. leucostictus*, introduced simultaneously, began to displace the former. Lowe-McConnell (1982) related such changes to the food supply.

De Silva et al. (1984) reported that *E. suratensis*, which feeds on molluscs in a lagoon, its original habitat, feeds on macrophytes in freshwater reservoirs. De Silva et al. (1984) found similarities in dentition of *E. suratensis* and *T. rendalli*, and that dentition of *E. suratensis* is well suited to molluscs and macrophytes. Mendis (1965) reported a general paucity of molluscs in inland reservoirs in Sri Lanka.

Of the five reservoirs only Mahagama exhibited a luxuriant growth of macrophytes, particularly *Ipomea aquatica*. The dominance of *T. rendalli* and *E. suratensis* in Mahagama reservoir may be due to the presence of this macrophyte. The other reservoirs are either deeper or have more frequent and wide fluctuations of water level and a higher drawdown and do not support luxuriant macrophyte growth. In these tanks *T. rendalli* and *E. suratensis* mainly depend on terrestrial vegetation, which covers the exposed areas at low water levels, and is important food after flooding. This paucity of macrophytes possibly limits the contribution of *T. rendalli* and *E. suratensis* to the fishery.

O. niloticus, a mouthbreeding cichlid, was introduced in 1975 (Fernando 1979), but concerted effort to stock it in the reservoirs was made only after 1978. The only reservoir stocked with appreciable numbers of *O. niloticus* in 1978-1979 was Soraborawewa (personal observation). *O. mossambicus*, which used to constitute 95% of the fish catch, declined to 5% and *O. niloticus* began to dominate the fishery in a short span of four years (Fig. 2a). Also, there was a dramatic increase in fish yield after the introduction of *O. niloticus*, from 239.4 t in 1978 to 787.3 t in 1982. This increase cannot be attributed entirely to increase in fishing effort as the catch/craft/month also showed a fourfold increase from 0.623 t in 1978 to 2.24 t in 1981 (Fig. 2 b).

Similar displacing by *O. niloticus* was reported by Lamarque et al. (1975) in Lake Itacy, Madagascar, where *S. macrochir* (introduced in 1958) prospered for several years before disappearing and was subsequently replaced by *O. niloticus* (introduced in 1961-1962). Welcomme (1966) reported that in Lake Victoria, *O. niloticus*, rarely in commercial catches before 1962, became the dominant species in some areas where *O. esculenta* was dominant earlier.

Phytoplankton samples collected in 1983 revealed that blue-green algae is the most dominant algae found in Soraborawewa, confirming the observations of Mendis (1965). *O. niloticus* mainly feeds on blue-green algae in Lake George, Uganda, and Moriarty and Moriarty (1973) demonstrated that *O. niloticus* could assimilate 70% to 80% of the carbon in ingested blue-green algae.

Costa and Abeysiri (1978) commented that *O. mossambicus* in Colombo Lake in Sri Lanka does not utilize blue-green algae, although these form an important component of its food. According to them *O. mossambicus* derives nutrition mainly from desmids and diatoms, so that large quantities of nutrients stored in the blue-green algae (which constitute a significant proportion of the total phytoplankton) are not utilized by the fish. Maitipe and De Silva (1984) found that blue-green algae played only a minor role in the diet of both adult and juvenile *O. mossambicus* in reservoirs. Dokulil (1983) reported a similar finding in Parakrama Samudra reservoir in Sri Lanka. Introduction of *O. niloticus* in Soraborawewa is likely to have resulted in positive results to the fishery, where a preferred and a utilizable food of *O. niloticus* existed in abundance.

Another important change in the species composition of Soraborawewa took place in 1983. As a result of a drought this reservoir dried up for about three months, December 1982-February 1983, leaving only a few pools of water. Once the reservoir filled up again, *O. mossambicus* became the dominant species while contribution of *O. niloticus* decreased to around 10%.

Two factors could have caused this change: (a) *O. mossambicus*, which is well established in almost all the freshwater bodies in Sri Lanka, could have gained access naturally through the various channels to Soraborawewa and (b) the superior ability of *O. mossambicus* to thrive under adverse conditions over *O. niloticus*. The contribution of *O. niloticus* to the fish catch increased again and reached 30% in 1984.

O. niloticus grows to a larger size than *O. mossambicus* in Soraborawewa and is preferred by consumers (personal observation). In Lakes Turkana and Albert in Africa, *O. niloticus* is larger growing and more abundant and in Lake Victoria, introduced *O. niloticus* grew much larger than the indigenous tilapias (Lowe-McConnell 1982). In Lake George, Uganda, *O. niloticus* contributes up to 80% of the catch by weight (Gwahaba 1973). *O. niloticus* exhibits a superior growth rate and attains larger sizes than *O. mossambicus* in seasonal tanks in Sri Lanka (Chandrasoma 1983). Fish catch of Handapangala reservoir (199.6 ha), Sri Lanka indicates that the contribution of *O. niloticus* to the fishery, only two years after its introduction, has reached 45%.

Mendis (1965) found blue-green algae in abundance in Sri Lanka reservoirs. Various workers (Dokulil et al. 1983; Chandrasoma et al., in preparation) reported that phytoplankton assemblage in various reservoirs in Sri Lanka is dominated by blue-green algae.

On the basis of the above observations it is recommended that a few selected perennial reservoirs in Sri Lanka be stocked with appreciable numbers of *O. niloticus* capable of utilizing blue-green algae found in abundance in Sri Lanka reservoirs to increase production from these fisheries.

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Table 1. Some features of the reservoirs

Reservoir	Surface area (ha)	Mean depth (m)	Conductivity at 25°C (µmhos)	pH (range)	Aquatic macrophyte vegetation
Udawalawe	3,454.0	20.3	179*	7.5* (7.3 – 7.6)	Scarce
Mahagama	168.0	2.8	460*	7.6* (7.2 – 7.8)	Abundant
Kiribbanarawewa	388.0	4.2	261*	7.8* (7.6 – 8.2)	Scarce
Chandrikawewa	447.0	8.5	183*	7.3* (7.0 – 7.7)	Scarce
Soraborawewa	670.0	4.0	200	7.3	Scarce

*From Maitipe and De Silva 1984

Table 2. Species composition (%) of fish catch of Kiribbanarawewa and Chandrikawewa reservoirs.

Reservoir	Year	*O. mossambicus*	*T. rendalli*	*E. surantensis*	Others
Kiribbanarawewa	1981	71.7	9.4	8.5	10.4
	1982	73.5	7.5	11.0	8.1
	1983	72.9	18.8	2.9	5.4
	1984	70.3	18.0	8.3	2.2
Chandrikawewa	1974*	84.0	–	5.2	10.8
	1981	75.1	5.8	7.7	11.4
	1982	71.8	12.5	6.7	8.7
	1983	71.1	15.3	7.3	6.3
	1984	75.5	6.6	8.8	7.8

*From Fernando 1974.

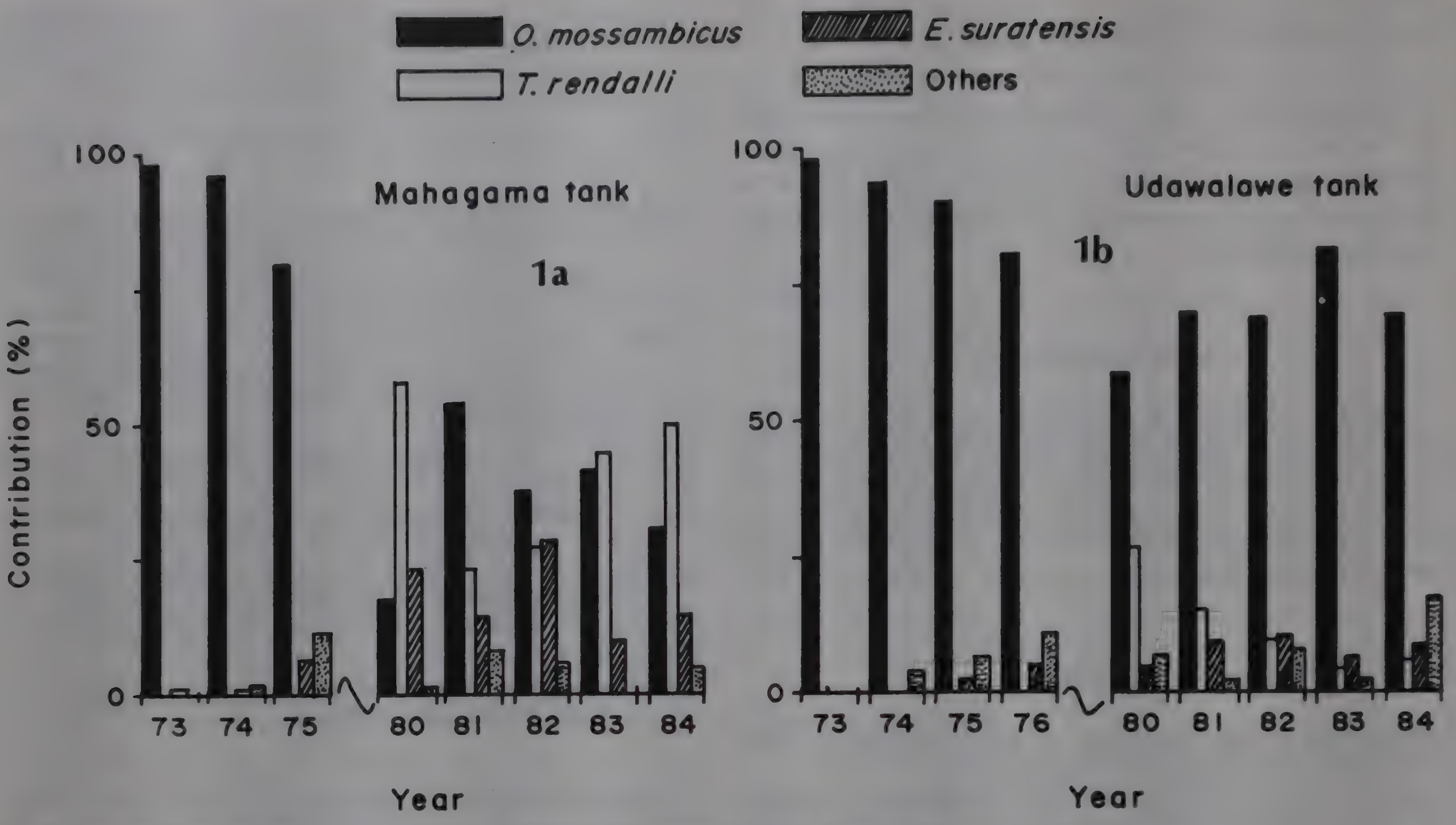

Fig. 1. Changes in the species composition of fish catches in Mahagama (1a) and Udawalawe reservoir (1b).

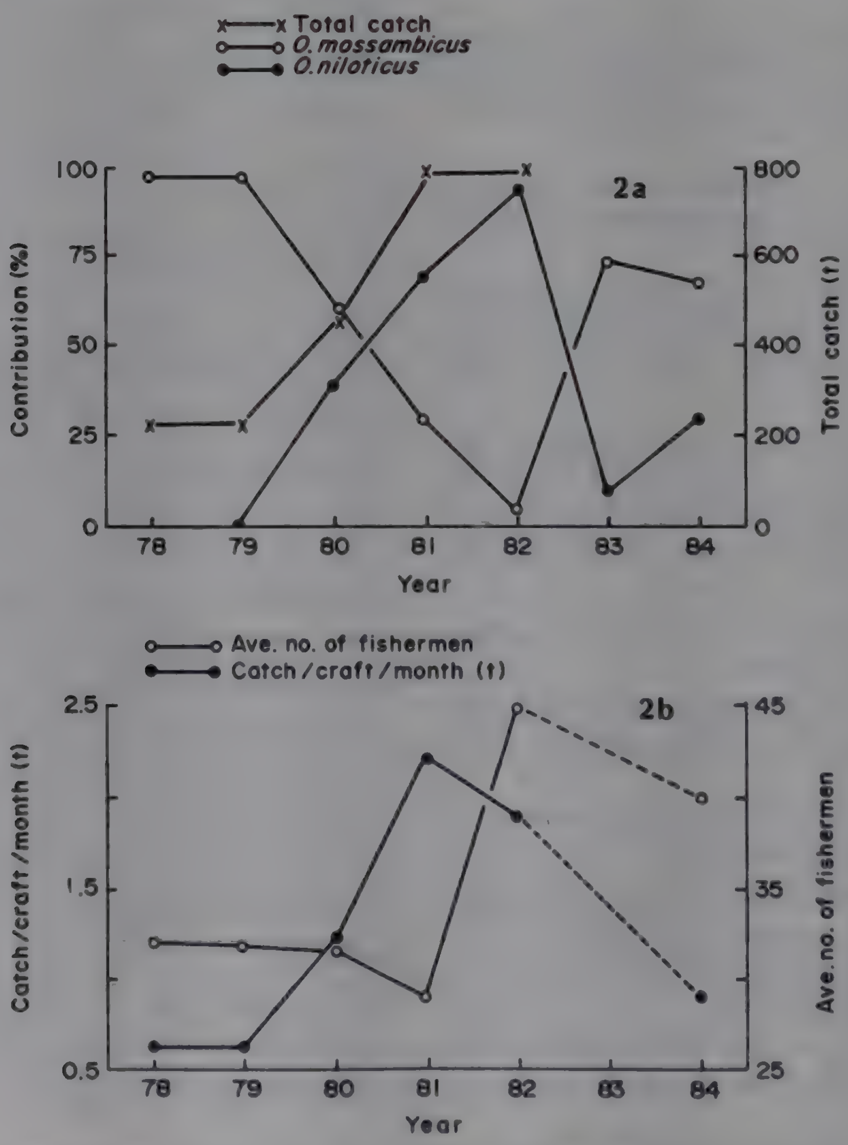

Fig. 2. Changes in the total catch and the species composition of Soraborawewa fishery (2a) and the changes in catch/craft/month and average number of fishermen (2b).

Development of Deep Sea Fishing in the East Sepik Province, Papua New Guinea

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Abstract

In 1983, a project commenced at Wewak, Northwestern Papua New Guinea to evaluate gear and vessel design for artisanal fishermen. The FAO-designed Samoan dropline was used to catch demersal fish on the outer reef slope in waters of 80-400 m depth. This method has now been accepted by local fishermen. Close monitoring has shown that as experience in the fishery increases so does catch per effort and economic return. Associated with the commercialization of this fishery has been the modification of traditional vessels and the introduction of new vessel designs. Although new designs were initially poorly received, their advantages became clear to the fishermen as they gained experience.

Introduction

Over the last 10 years, the South Pacific Commission (SPC) has encouraged many Pacific Island countries to develop artisanal fisheries which exploit demersal finfish resources associated with outer reef slopes. Crossland and Grandperrin (1980) reviewed the work of the SPC Deep Sea Fishing Development Project (DSFDP) which visited eight countries including Papua New Guinea (PNG).

The DSFDP visited PNG in 1979 to encourage bottom fishing in deep waters (80-400 m) along the outer reef slope. The project fished on the West New Britain coast (Fig. 1) where encouraging fishing trials were completed. As a result of this survey, the Fisheries Division of the PNG Department of Primary Industry (DPI) commenced pilot projects in 1983, aimed at developing deep sea artisanal fisheries using proven fishing methods, gears and suitable small-scale fishing vessels.

One such pilot project has operated in the East Sepik Province since 1983. This paper reports the findings of this project which had objectives of describing the nature of the demersal finfish resource in waters 80-400 m deep on the outer reef slope, assessing the suitability of various sea craft as a fishing platform, and of reporting the results of experimentation with various fishing gears.

Methods and Materials

The project is based at Wewak, East Sepik Province and has concentrated field work in the area known as Turubu. Travelling distances between Turubu fishing villages and the fish purchasing depot based at Wewak, range from 30 to 50 km. The distances to the fishing grounds from the fishing villages range from 7 to 19 km. In the Turubu area the sea bed is gently sloping, with a fine muddy substrate and sparse coral patches due to its proximity to the mouth of the Sepik River.

It has been demonstrated by DSFDP master fishermen in many Pacific Islands that the FAO-designed Samoan wooden handreel is a suitable fishing gear for harvesting deepwater snapper (Crossland and Grandperrin 1980).

Local outrigger canoes were supplied with three handreels each containing 300-400 m of 68-136 kg breaking strain monofilament nylon line. The line terminated at a swivel connected to 1-1.5 m of 1-mm diameter multistrand wire trace with three tuna circle hooks (No. 39960 ST; size 5, 6 and 7 with a terminal weight of 1.5-2 kg).

Because of the relatively unseaworthy nature of the local outrigger canoes, local fishermen fish on a day-trip basis. Depending on the weather conditions one to four such fishing trips can be made each week. Two trips per week are normally made to the fish depot to sell the catch and to pick up fuel, ice and supplies. The project fished on an extended basis and completed one or two trips per week, each trip lasting 2-4 days.

Five different small-scale fishing vessels were used in the project. These were: 4-m aluminium dinghies, 11-m local single outrigger canoes, an 11-m FAO designed plywood, outrigger canoe, an 8.3-m Alia-catamaran and a 7.3-m Sandskipper-style catamaran.

Four of these vessels were powered by outboard motor engines of 15-25 hp. The Sandskipper was propelled by a long tail 13-hp diesel engine. The Turubu fishermen used local outrigger canoes. Each canoe carries a wooden ice box capable of carrying 200-300 kg of ice and produce.

Results

Fig. 1 shows the annual landings of finfish from East Sepik Province from 1978 to 1984. There was a significant decline in annual catch from 1979 to 1982. Catches started to increase from 1983 after the introduction of deep sea fishing to the Province. Most of the increase was in the deep sea fishery.

The deep sea catch contributed 57% while surface trolling contributed 13.5%, and combined gill net and handline, 24.1%. The number of fishermen each month varied from one to 15 during 1983-1985 with no seasonal pattern.

It was hypothesized that local outrigger canoes are seaworthy when used for extended deep sea fishing trips and relatively cheap to operate when powered by outboard motors. Analysis of variance (ANOVA) was used to determine the difference in fuel consumption between vessel types using 25-hp outboard motors. Data on fuel consumption for five vessel types were compared. Fuel consumption showed a significant difference ($p < 0.05$) between local outrigger canoes powered by a 25-hp outboard motor and introduced vessels, the Alia-catamaran and the improved FAO plywood outrigger canoe powered by the same outboard. The introduced outrigger canoe using a 15-hp outboard motor was found to be less expensive on fuel than the local outrigger canoe.

Similar tests were carried out using ANOVA to determine the difference in fuel consumption rate between 15-hp and 25-hp engines. It was found that 15-hp petrol outboard motors use less fuel than 25-hp models on the same hull types ($p < 0.01$).

Fishermen are responding favorably toward the introduced technology. It has been reported that several fishermen have sold their 25-hp outboard motors for 15-hp motors. The lengths of the local outrigger canoes entering the fishery are decreasing from 11 m to 8 and 9 m due to the ability to beach smaller canoes.

Local outrigger canoes were hypothesized to be seaworthy and to be able to fish all year-round irrespective of wind and weather conditions. A chi-square test used to determine the frequency of fishing trips in varying wind strengths found a highly significant difference ($p < 0.01$). Fishermen using local outrigger canoes were found to fish when the maximum wind strength was less than 10 knots. The results, however, do not necessarily mean that the local vessels are not seaworthy.

Catch per effort has been increasing since the project began as fishermen gained experience. In the study area, average catch in 1983 was 34.5 kg/canoe day; in 1984 it was 41.4 kg/canoe day; and in 1985, 48.5 kg/canoe day. Similar increases were observed in nearby areas where the technology was being adopted.

Table 1 shows generic composition of the Turubu fishery. From September 1983 to September 1985, 105 species of deep sea fish belonging to 29 genera representing 13 families were caught. Six species from the Lutjanidae and one species from the Carangidae represented 80.7% of the total saleable catch. The fish are purchased from fishermen in two grades (A and B) according to market preference. Grade A accounts for 72% of the deep sea catch.

Discussion

The demersal stocks of the outer reef slope in PNG represent a virgin resource that has, as a result of the 1979 DSFDP survey in West New Britain, attracted the attention of local fisheries workers. However, only at Wewak have local fishermen actively exploited this resource with the result that now there is a rapidly developing commercial fishery targeting deepwater snappers in the area.

The results of the survey among the fishermen suggested that the size of the snapper resource is large enough to sustain the current level of effort as there has been a steady increase in catch with effort since the commencement of fishing.

In order to develop further this resource, there is a need to improve the seaworthiness of the local outrigger canoes. Plans have been drawn up by FAO (Gulbranson, pers. comm.) for an improved version of the present FAO 11-m plywood canoe.

When suitable small-scale fishing canoes are available in the fishery, fishermen will have to be trained to fish more effectively with the fishing gear and build up knowledge of their fishing grounds. It has been shown that as new fishermen become engaged in deep sea fishing, the catch rate is initially low and increases with experience. Turubu experienced fishermen have now learnt to target high priced fish species such as the red snapper. This improves the economic return for the effort.

The current trend in the fishery indicates that by 1987, the number of experienced fishermen will more than double. Important questions, such as the size of the finfish resource, the robustness of the target species to constant fishing, seasonality of the fishery, and the ability of the local market to support this fast growing fishery will need to be answered.

Acknowledgements

I would like to express my gratitude to S.D. Frusher for his valuable assistance and comments on the manuscript and Mr. E. Tamba for compiling the data.

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Table 1. Species composition by generic taxa group for the Turubu fishery.

Genus group	No. species	Total individuals	% no.	Total weight (kg)	% by wt.
Pristipomoides	5	3,517	37.8	5,725.18	35.8
Lutjanus	17	2,329	25.0	4,071.24	25.5
Etelis	2	337	3.6	572.21	3.6
Macolor	1	6	0.1	16.68	0.1
Aphareus	1	68	0.7	187.44	1.2
Aprion	1	1	0.1	3.7	0.1
Paracaesio	2	29	0.3	35.15	0.2
Pinjalo	1	373	4.0	711.62	4.5
Carangoides	7	214	2.3	217.54	1.4
Caranx	6	1,768	19.0	3,072.83	19.2
Longirostrum	1	1	0.1	1.6	0.1
Seriola	1	65	0.7	231.54	1.5
Ephinephelus	19	228	2.5	485.33	3.0
Cephalopholis	8	20	0.2	13.27	0.1
Variola	2	8	0.1	3.4	0.1
Lethrinus	9	138	1.5	254.76	1.6
Gymnocranius	4	33	0.4	56.85	0.4
Wattsia	1	38	0.4	42.81	0.3
Sphyraena	2	64	0.7	165.91	1.0
Argyrops	1	20	0.2	47.77	0.3
Nemipterus	2	8	0.1	4.09	0.1
Branchiostegus	3	8	0.1	5.43	0.1
Plectropomus	2	8	0.1	14.98	0.1
Ostichthys	1	2	0.1	0.4	0.1
Arius	3	13	0.1	16.33	0.1
Pomadasys	3	8	0.1	4.4	0.1
Odonus	1	1	0.1	0.3	0.1
Gymnosarda	1	1	0.1	5.93	0.1
Cheilinus	1	1	0.1	1.20	0.1
Total (29)	105	9,309		15,974.5	

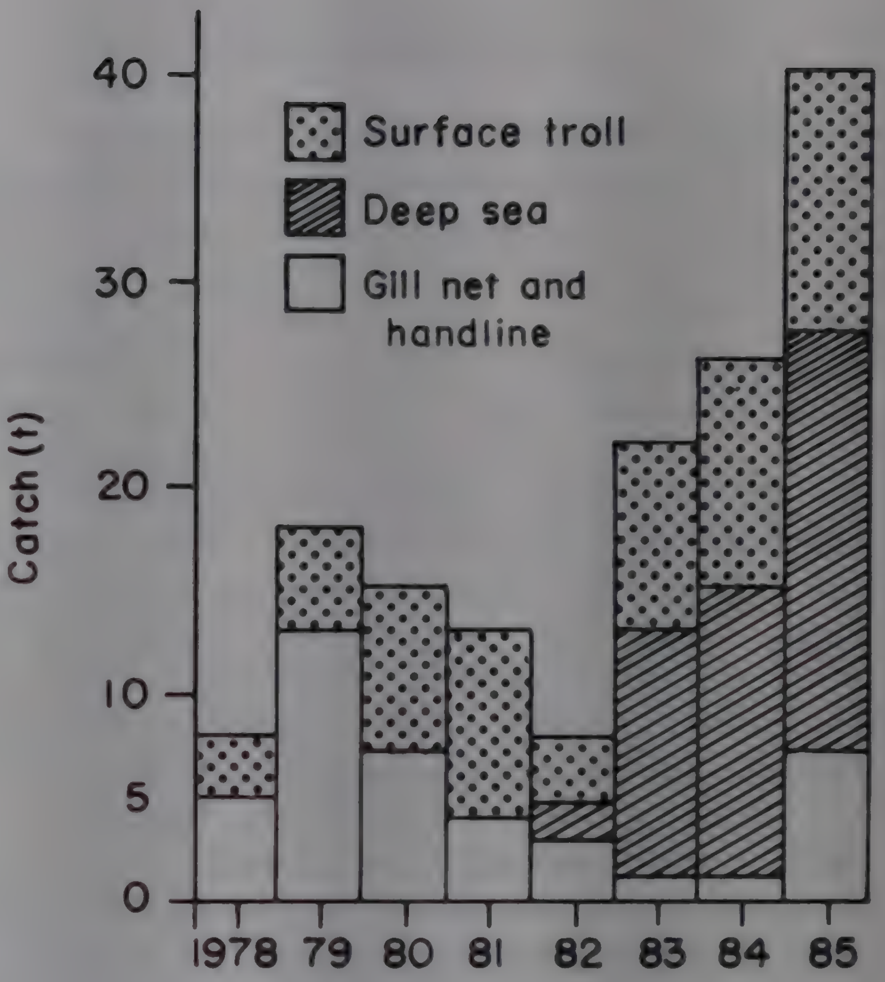

Fig. 1. Annual catch in East Sepik Province, Papua New Guinea, by gear type.

Effects of the Urchin Fishery on the Population Structure of *Anthocidaris crassispina* (Echinodermata: Echinoidea) in Hong Kong

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CHIU, S.T. 1986. Effects of the urchin fishery on the population structure of *Anthocidaris crassispina* (Echinodermata: Echinoidea) in Hong Kong, p. 361-366. *In* J.L. Maclean, L.B. Dizon and L.V. Hosillos (eds.) The First Asian Fisheries Forum. Asian Fisheries Society, Manila, Philippines.

Abstract

Anthocidaris crassispina is the only economically important sea urchin species harvested in Hong Kong. Fishing pressure is especially intense on the eastern and southern coasts of the territory. The major spawning season was found to occur in autumn (October-November). The existing fishing season is from January to April and coincides with the active feeding period when gonads build up nutrient reserves for gametogenesis. Population studies confirmed that fishing activities remove the larger animals over 45-mm test diameter. Despite this, substantial recruitment is recorded annually in heavily fished areas in the south and east. In the sparsely populated southwestern quadrant of Hong Kong, there is only minimal fishing, a predominance of larger animals (over 50-mm test diameter) but no recorded juvenile recruitment. Salinity fluctuations in this estuarine area are postulated to be limiting. The overall decline in urchin population and the fishery is attributed to the destruction of suitable habitats due to extensive coastal reclamation and pollution.

Introduction

Anthocidaris crassispina (A. Agassiz) is the only sea urchin species of economic importance in Hong Kong. Fishery catch statistics have been kept since 1975 (Thompson 1982), and these have shown a progressive decline in the volume of the catch since 1981.

Changes in environmental conditions, such as pollution, leading to a decrease in suitable substrate or food, are possible causes of the decline. On the other hand, the depletion or elimination of urchin populations by overexploitation has been well documented (Southward and Southward 1975; Bernard 1977).

Information on the population ecology of *Anthocidaris crassispina* is limited (Thomson 1982; Yoo et al. 1982). It was therefore not possible to postulate whether or not the decline in urchin populations and the industry is due to overfishing or to other changes in the environment. Information on population structure, recruitment rates and the abundance of commercial-sized urchins in various parts of Hong Kong waters are important for the assessment of the potential for the urchin roe industry.

Study Areas and Methods

A general study of urchin distribution was conducted throughout the Hong Kong coastline. Four study sites (Fig. 1) were selected for a comparative study of the *A. crassispina* populations, based on colony size (Table 2), hydrographic conditions (Table 1), seasonal food availability (Chiu 1985), human activities and degree of fishing pressure (Table 1). Gemini Beach marks the westernmost limit of *A. crassispina* occurrence.

The study period extended from January 1983 to December 1984. Sampling by SCUBA diving was carried out at monthly intervals. Preliminary line-transect studies at each station delimited areas with highest urchin abundance. Depending on the density of the animals, an area with an average of 50-70 urchins was demarcated (Table 2), and all the animals within it collected. Boulders were upturned and crevices examined to locate the juveniles. Test diameter and height were measured to the nearest 0.1 mm with Vernier calipers, and wet weight to the nearest 0.1 g. Apart from subsamples of different-sized urchins (at 5-mm test diameter class intervals) collected for gonad studies, all individuals were returned to the collection site. Specimens were preserved in 40% formaldehyde.

In the laboratory, urchins were dissected and the dry weights of the gonads, test and viscera were obtained by drying at 60°C for 72 hours. To minimize variations in gonad weight with size (Gonor 1972), the gonad index was calculated for animals greater than 40-mm test diameter. The formula is as follows:

Gonad Index = Dry weight of gonads/Total dry weight of urchin x 100.

Results

Destruction of urchin habitat. A. crassispina is a benthic grazer of the shallow sublittoral zone of rocky shores in Hong Kong. In a survey conducted from 1977 to 1980, Thompson (1982) reported that urchins were abundant on bedrock surfaces, at densities ranging from 0.2 to $19.4m^2$. This study indicates that comparable densities are still recorded for most well-established colonies (Table 2). The major difference, however, is that there is a decrease in the number of colonies, in particular along the shores of Tolo Harbour and Channel in northeastern Hong Kong (Fig. 1).

The *A. crassispina* fishery extends from Crooked Harbour in the northeast to the Soko Islands in the southwest but has traditionally been centered upon the northeast (Tolo Harbour and Channel) and the eastern (Port Shelter and Rocky Harbour) quadrant.

Since 1980, extensive foreshore reclamation for urban development has occurred within Tolo Harbour on virtually all of the northern, southern and western shores within this inlet. Reclamation has increased the silt loads of the waters of Tolo Harbour and Channel (Morton 1982) which adversely affects algal growth. This has significantly reduced the available and suitable rocky substrates for the urchins. Hence, the decrease in urchin colonies on the formerly well-populated northeastern coastline. *A. crassispina* has never been established in the western parts of Hong Kong (Fig. 1) (Chiu 1985).

Population structure. The size and characteristics of *A. crassispina* colonies differ at the four sites (Table 1). Of the three sites on the south and the east, Cape D'Aguilar has the largest colony although a disease outbreak in June-July 1983 decimated almost 80% of the population. Only a small colony of urchins is, however, present at Gemini Beach.

Analysis of the size-frequency distributions (Fig. 2 A-D), using the methods of Harding (1949), indicates that the three large urchin populations are polymodal. At Lung Ha Wan and St. Stephen's Beach, urchins range from 5 to 70 mm test diameter, with 2 modes at 15-30 and 40-50 mm. Two similar size groups dominated the population at Cape D'Aguilar before the disease outbreak. There was mass mortality of the smaller animals, and recovery took approximately 10 months. During the two consecutive years of study at the three sites, juvenile settlement was recorded from February to June. The influx of juveniles constituted 9-17% of the population. Larger urchins exceeding 55 mm were seldom found, as intensive harvesting of individuals greater than 45 mm occurs from January to April.

Gemini Beach in west Hong Kong has a distinctly different population. The size-frequency distribution has been shown to be consistently unimodal, with the majority of urchins (54.0-90.5%) in the 50-65 mm size range. No juvenile recruitment was observed during the study period, and a fishery does not exist here because of low urchin densities and poor underwater visibility year-round.

Gonad development. The gonad index (Fig. 3) indicates a well-defined annual cycle of gonad development. In general, the gonads progressively increased in weight from December or January to attain maximum values by April/May. The indices then declined to low values in October/November, suggesting that spawning had occurred during this period. The cycle is essentially similar at all stations, but the indices were on the average higher in the second year (1984).

Despite the differences in population structure, the colony of larger urchins at Gemini Beach exhibited the same seasonal gonad development cycle and the gonadal indices are comparable with the other three stations.

Discussion

Aside from human activities, food and salinity exert important influences on natural urchin communities in Hong Kong.

Annual intraspecific variation in gonad growth has been attributed to habitat and food related differences (Ebert 1968; Keats et al. 1984). In Hong Kong, phaeophytic macroalgae, the preferred food of *A. crassispina* (Chiu 1985), shows a strong pattern of seasonal occurrence and abundance (Hodgkiss 1984). This is reflected in seasonal variations in the urchin diet (Fig. 4), and subsequently structures the gonad development cycle. *A. crassispina* becomes sexually mature upon reaching 15 to 20 mm test diameter. Intensive feeding during peak algal abundance (January-May) results in a buildup of nutritive phagocytes in the gonads (Chiu, unpublished data) and an increase in gonad weight is recorded. The animals are ready to spawn from July to November, after which the spent gonads shrink and the feeding cycle starts again. Absence of suitable and adequate food would therefore limit the development of well-established urchin colonies.

In western Hong Kong, in addition to a lack of suitable food, wide salinity fluctuations become more important to the urchin. Thompson (1983) has shown that when urchins are under nutritive stress, gonad output is maintained at the expense of somatic production. This appears to be the case at Gemini Beach where the urchins maintain apparently normal gonadal development. However, juvenile recruitment had been unsuccessful for at least the two consecutive years of study. Echinoderms are generally considered stenohaline marine invertebrates intolerant of salinity fluctuations (Binyon 1966). When exposed to environmental stress, their colonies become patchy and recruitment is sporadic with populations in estuarine areas dominated by large individuals (Drouin et al. 1985). The urchin colony at Gemini Beach is therefore typical of an estuarine population at the limit of its hyposalinity tolerance. The heavy freshwater discharge from the Pearl River often causes salinity levels to fall below 15 ppt at the western approaches of Hong Kong. Heavy mortality could be expected as salinity tolerance experiments on *A. crassispina* show that small urchins (< 20 mm) become moribund at salinities below 20 ppt (Chiu, unpublished data). Successful recruitment at Gemini Beach is therefore not expected to be regular, and *A. crassispina* colonization of the areas to the west of Gemini is also precluded due to yet lower salinities.

Where food and hydrographic conditions are not limiting, *A. crassispina* populations are still affected by human activities. Recreational activities affect certain populations. At Cape D'Aguilar, where access by road is restricted, a large population is maintained; but at popular swimming beaches like Lung Ha Wan and St. Stephen's Beach, urchins are frequently removed by swimmers and divers year-round. Intensive harvesting by fishermen has a more severe impact. It is estimated that fishermen remove between 10 and 20% of the total population and between 10 and 35% of the reproductive population. Acceptable fishing rates have rarely been determined for sea urchin populations, but the rates in Hong Kong are low when compared with annual rates of 41 to 78% reported from Japan (Kawamura 1974). This study also shows that the remaining population still comprises a high percentage of smaller but sexually mature urchins, and that the magnitude of annual recruitment is approximately equivalent to adult fishing mortality. Hence, the existing harvesting strategy does not appear to be detrimental to the total *A. crassispina* population.

By far the most important factor contributing to the overall decline of the *A. crassipina* population in Hong Kong is the dramatic decrease in suitable urchin habitats. Extensive foreshore reclamations of the coastline which has traditionally supported an urchin fishery, with increased pollution and silt loads, have removed both suitable substrates and algal food.

Despite evidence of intensive fishing, it is concluded that pollution through habitat destruction is the major factor limiting *A. crassispina* in Hong Kong. It is envisaged that if further depletion of the urchins from these pressures occurs, this will eventually result in the collapse of the commercial fishery.

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Table 1. Characteristics of *A. crassispina* study sites.

Study site	Salinity (ppt) Temperature (°C)	Macroalgae occurrence	Fishing pressure	Remarks
Lung Ha Wan	29.0 – 35.0 15.5 – 30.5	All year round	Heavy	SCUBA diving training area
Cape D'Aguilar	20.0 – 36.0 14.5 – 29.5	December-June	Heavy	Restricted access
St. Stephen's Beach	27.0 – 35.0 13.8 – 29.5	December-June	Heavy	Very popular public swimming beach
Gemini Beach	15.0 – 36.0 13.5 – 29.5	January-February (mainly drift algae)	Nil	Not popular public swimming beach

Table 2. Sizes of *A. crassispina* colonies at sites.

Site	Estimated no. of urchins	Area (m^2)	Average density (no. of urchins/m^2)	Predetermined sample area (m^2)
Lung Ha Wan	14,000	3,600	3.8	15 x 15
Cape D'Aguilar	176,000 (before July 1983) 28,000 (after July 1983)	14,000 14,000	12.7 2.0	5 x 5 20 x 20
St. Stephen's Beach	15,000	5,000	3.0	10 x 10
Gemini Beach	2,500	2,400	1.0	10 x 30

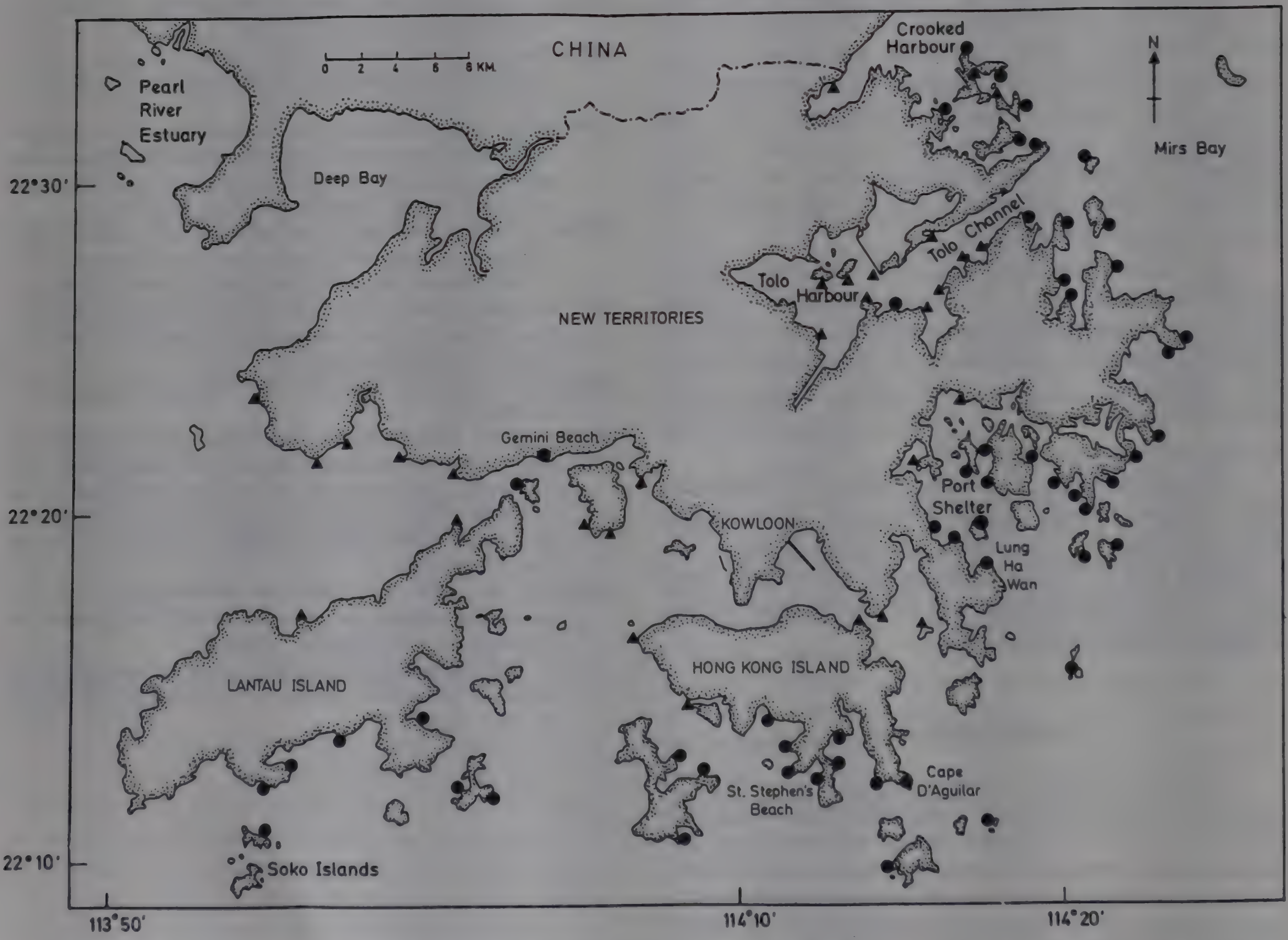
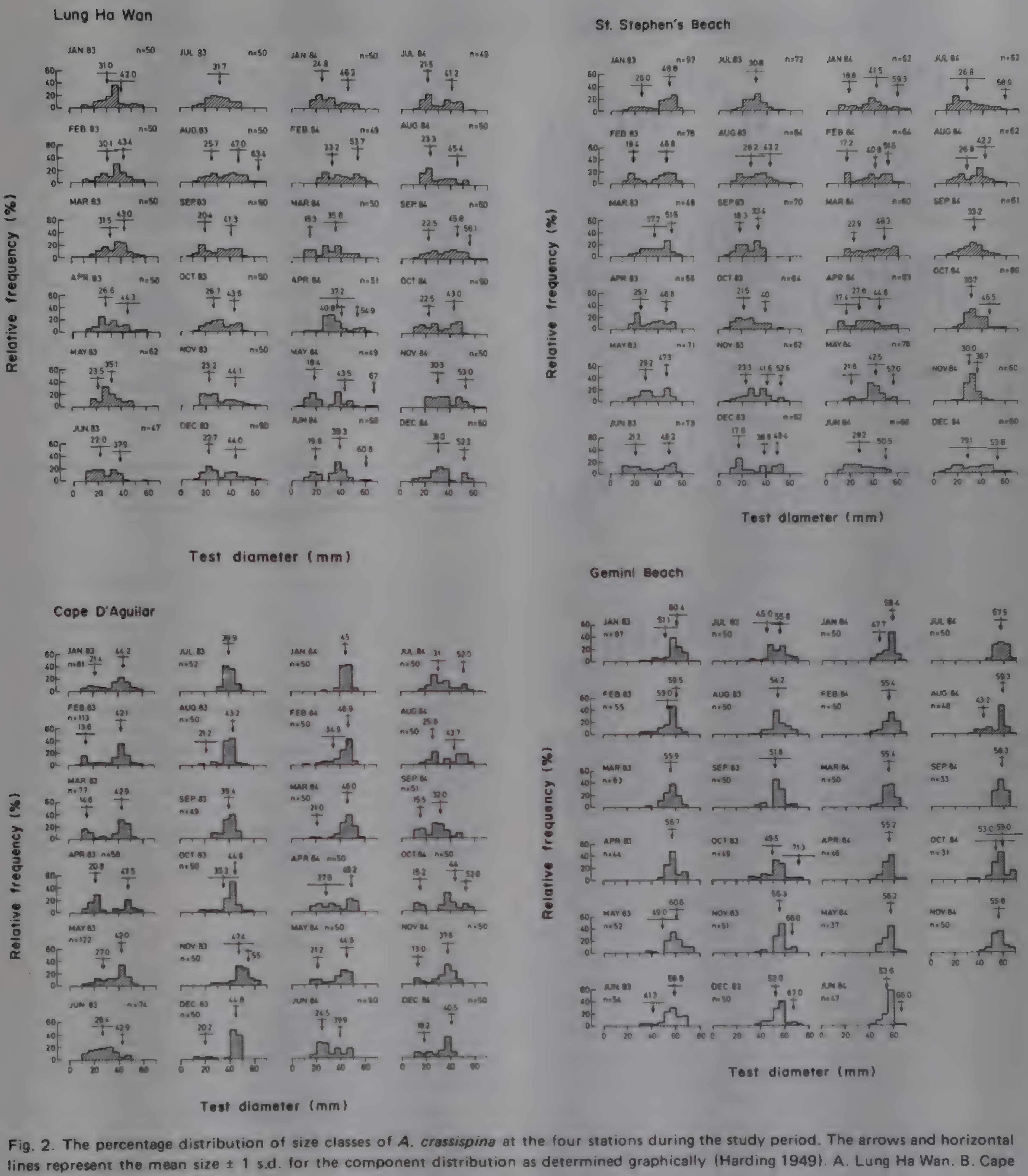

CHINA
0 2 4 6 8 KM.
Pearl River Estuary
Deep Bay
Crooked Harbour
N
Mirs Bay
Tolo Channel
Tolo Harbour
NEW TERRITORIES
Gemini Beach
KOWLOON
Port Shelter
Lung Ha Wan
LANTAU ISLAND
HONG KONG ISLAND
St. Stephen's Beach
Cape D'Aguilar
Soko Islands
22°30'
22°20'
22°10'
113°50'
114°10'
114°20'

Fig. 1. Map of Hong Kong showing the study areas and the distribution of *A. crassispina*. Closed circles and triangles respectively denote the presence and absence of *A. crassispina* population.



Lung Ha Wan

St. Stephen's Beach

Cape D'Aguilar

Gemini Beach

Fig. 2. The percentage distribution of size classes of *A. crassispina* at the four stations during the study period. The arrows and horizontal lines represent the mean size ± 1 s.d. for the component distribution as determined graphically (Harding 1949). A. Lung Ha Wan. B. Cape D'Aguilar. C. St. Stephen's Beach. D. Gemini Beach.

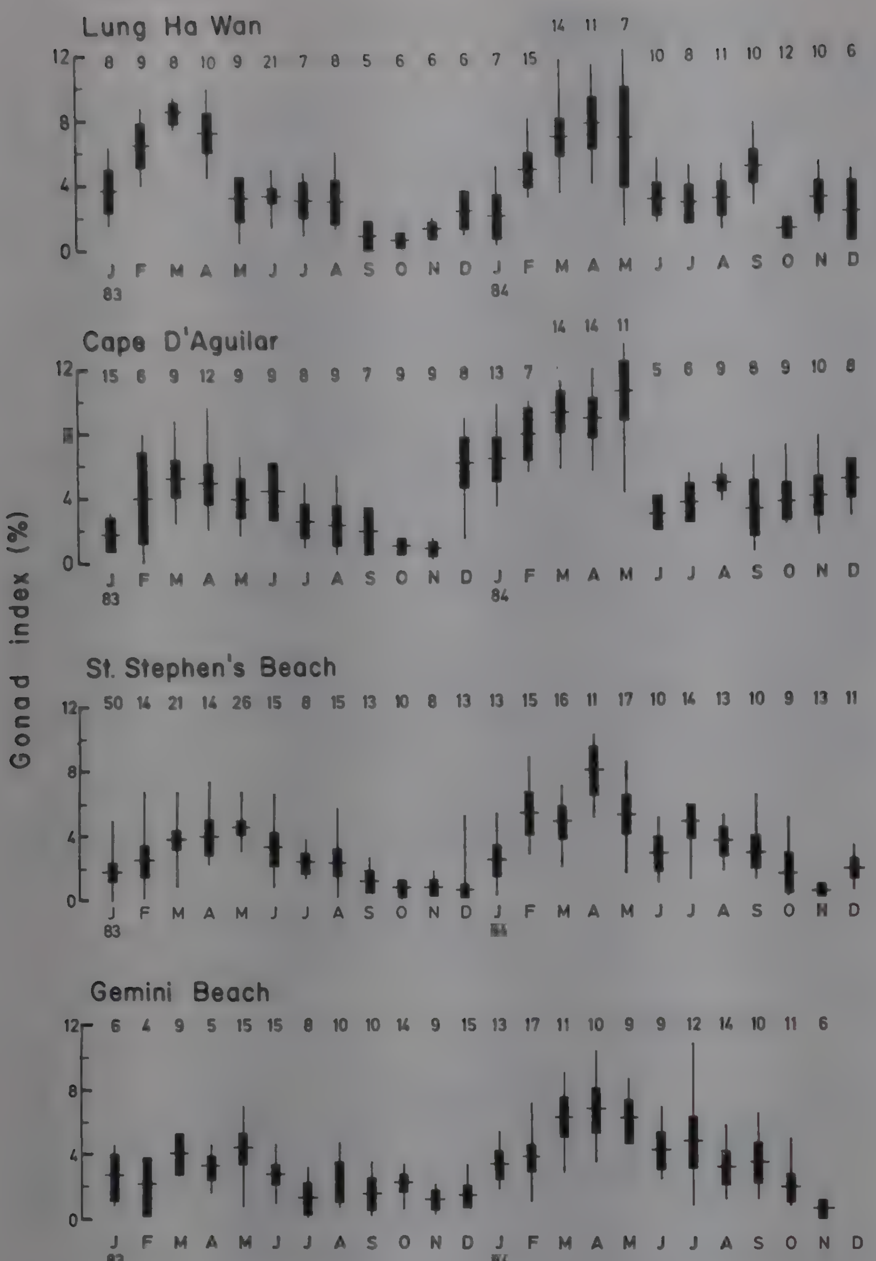


Fig. 3. The seasonal variation in gonad indices for *A. crassispina* at the four study sites. The horizontal lines denote the mean monthly gonad indices. Vertical bars show the 95% confidence limits and vertical lines are the range of the gonad index values. N is the number of animals in each sample.

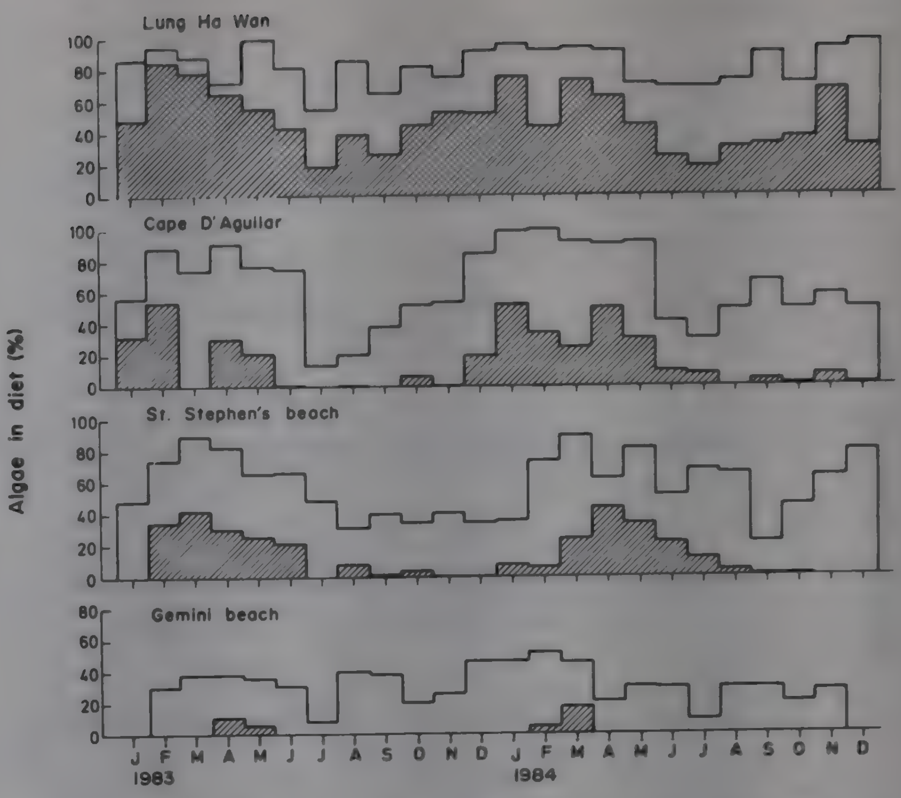


Fig. 4. The seasonal variation of the macroalgal component in the diet of *A. crassispina* at the four study sites. Hatched areas represent the percentage of phaeophytic macroalgae.

Fisheries Development of the Sepik River, Papua New Guinea: Proposed Fish Introductions

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COATES, D. 1986. Fisheries development of the Sepik River, Papua New Guinea: proposed fish introduction, p. 367-370. *In* J.L. Maclean, L.B. Dizon and L.V. Hosillos (eds.) The First Asian Fisheries Forum. Asian Fisheries Society, Manila, Philippines.

Abstract

The Sepik is Papua New Guinea's largest river system and supports an important subsistence fishery. Estimates have shown that the yield of the fishery is less than 10% of that expected by comparison with rivers in other continental regions. People inhabiting the Sepik catchment suffer serious problems of protein malnourishment. Over half of the present catch is introduced tilapia. The main reason for the low yield is the inadequacy of the native ichthyofauna from the fisheries point of view: for zoogeographic reasons, families of freshwater fish composing catches from other regions do not naturally occur in Australasia. Serious problems are envisaged if attempts are made to improve the yield based on native species. The improvement of the fish stocks by species introductions has been proposed. The procedure being adopted to ensure adequate safeguards is outlined. Each species considered will be appraised for its potential benefits and risks before final decisions on introduction are made. Assistance, advice and comments from scientists with relevant experience within the region are sought.

Introduction

In a recent review of floodplain river fisheries Welcomme (1979) made no significant mention of the New Guinea region. This is a testament to our previous lack of knowledge of this region. The island has three of the world's largest rivers: the Fly in the south and the Mamberambo/Idenberg and Sepik Rivers in the north.

The fisheries yields of floodplain rivers in Asia, South America and Africa are broadly comparable (Welcomme 1976, 1979). The systems are similar and have ichthyofaunas that are long established and that show marked ecological convergence (Lowe-McConnell 1975). New Guinea, however, is part of the Australasian region and primary freshwater fishes or long-established secondary freshwater fishes are lacking from the fauna. The region is east of "Wallace's Line" (Fig. 1), a faunistic barrier between Papua New Guinea (PNG) and Southeast Asia, as explained further by Coates (1985). Most native fishes found in freshwater in New Guinea are either diadromous (e.g., Megalopidae, Lutjanidae, Gobiidae) or freshwater representatives of essentially marine groups (e.g., Ariidae, Melanotaeniidae, Eleotridae). A similar situation occurs in Australia and New Zealand, and several other smaller areas. Within this region only the island of New Guinea has substantial freshwater resources that need to be exploited by people for subsistence food and as their sole major potential source of income.

Coates (1985) estimated the yield of the floodplain of the Sepik River system to be between 3,000 and 5,000 t/year. This is approximately 10% of the yield predicted with, for example, African rivers. About 50% of the present catch is composed of introduced tilapia *(Oreochromis mossambicus)*. Considering the native species alone the present yield of the fishery is only about 5% of that predicted. Fishing effort may be low by comparison with other regions but the low yield is mainly due to the depauperate native freshwater fauna.

One obvious method of improving the Sepik fishery is to introduce further fish species. A lengthy discussion of the rationale for fish species introductions is presented in Coates (in press). The present paper serves to summarize the situation and explain the procedure being adopted.

The introduction into and distribution of "exotic" fishes in PNG has been summarized by West and Glucksman (1976). Three species have established in the Sepik. The mosquitofish *(Gambusia affinis)* was introduced intentionally in the 1930s and has produced large populations in the Sepik, although its benefits for mosquito control are not known. Common carp *(Cyprinus carpio)* and tilapia were both brought to Papua New Guinea originally for aquaculture. Tilapia "accidentally" escaped from ponds in the drainage system in the early 1960s and established wild populations in the Sepik; tilapia is now the most important species in the fishery. Common carp escaped from ponds stocked in the highlands and entered the Sepik floodplain in 1979. Carp are still spreading but in regions where they are established species have produced large and valuable populations (Coates 1984).

These past introductions illustrate that fish species properly adapted to the Sepik do well and produce large and useful populations. They also illustrate that past introductions have been very haphazard and insufficient attention was given to appropriate procedures, possible effects on the native fauna and risk/benefit analyses. No problems are known to have occurred.

The Sepik River System

Over 84% of PNG's population live inland and have no access to marine resources (Frielink 1983). Over 99% of PNG's fishermen are subsistence fishermen. The inland freshwater resources are centered on the major river systems. The Sepik River (Fig. 1) is PNG's largest river and supports an important subsistence fishery due to the relatively large population living there, although the yield is low (Coates 1985). The limnology of the region has been studied by Coates et al. (1983). Nutrient levels are comparable to other similar river systems.

Recent nutritional surveys have indicated that in many areas of the Sepik over 50% of children below the age of five years are malnourished (by PNG adopted definitions), in some areas the incidence is as high as 75%. The main problem is protein malnourishment.

The Sepik floodplain fishery is broadly composed of: tilapia (about 50%); eight species of fork-tailed ariid catfishes (about 25%); two eleotrids *(Oxyeleotris lineolatus* and *Ophieleotris aporos)* (about 25%); tarpon *(Megalops cyprinoides)* (less than 5%); and less than 2% of all other species.

Less is known about the hill stream regions (away from the floodplain) except that the fish fauna is poorer still in terms of species abundance and biomass. Ariid catfishes and tilapia are absent from these areas and eleotrids, melanotaeniids and theraponids become relatively more important.

The main reason for low yields is probably the lack of species adapted to exploit floodplain conditions. The only species producing significant populations on the floodplain are the two eleotrids and the rainbowfish *Glossolepis multisquamatus* which is too small to be of importance to the fishery. The ariids are mainly restricted to the rivers. The majority of fish production in such rivers normally occurs on the floodplain (Welcomme 1979). The success of tilapia in the river system is attributed to its ability to exploit floodplain conditions. Tilapia grow rapidly, have a high reproductive rate and most of the production occurs on the floodplain during the flood.

A further problem with the native species is that most have very low fecundity. The ariids, for example, produce a maximum of 175 eggs per spawning from fish about 1.0 m total length (Coates 1983). Most ariids produce 15-25 eggs per spawning. Only the two eleotrids and one rainbowfish mentioned above have a high reproductive rate. Most native Sepik fishes are, therefore, not adapted to cope with increases in mortality of adults and would probably be quickly overfished if fishing was intensified. This has already happened with Sepik ariids in some areas. It is well known that as tropical multispecies fisheries become fished the older, slower growing fishes with low reproductive rates tend to become replaced by younger, faster growing species with higher reproductive rates (Lowe-McConnell 1975; Welcomme 1979). The problem in the Sepik is that if the former are removed by increased fishing then there are few species in the latter category that could replace them. In other words, a substantial increase in yield based on native species could not be maintained.

Alternatives to Species Introduction

The basic objectives of fisheries development in PNG are to promote cash earning opportunities by rational exploitation of renewable fisheries resources, and to improve the subsistence diet and nutritional status of the people. Before species introductions to achieve these goals can be considered the alternatives should be investigated. These are:

Transport fish from coastal to inland areas. The coastal resources of PNG are quite rich. It is possible to catch fish at the coast and transport them inland. However, the coastal fishery cannot at present provide fish efficiently and economically to coastal people let alone cover the cost of transportation inland. The majority of inland people have no money with which to buy fish from elsewhere.

Develop the fishery based on existing species. Some improvements could be made in the present yield by increasing fishing effort and introducing new fishing and preservation techniques. However, as explained above, because of the problems with the native fauna, any improvements along these lines would be modest and at considerable cost.

Aquaculture. There are few regions suitable for aquaculture and few, if any, waste foods to feed fish. The amount of fish that could be produced would not rival that which could be obtained via fishing new wild stocks. Aquaculture has never been a success anywhere in PNG, mainly due to the inexperience and lack of motivation of the people. Aquaculture would need to be based on introduced species since none of the species presently available are of proven use.

Do nothing. If this option is taken then the government and the fisheries department are ignoring their main objectives and obligations. They will be denying the Sepik people of a cheap and large resource available through species introductions.

Potential risks vary with the species considered as do potential benefits. There are two distinct dilemmas:

First, there will never be enough information available on the Sepik or on the species proposed for introduction, to be able to predict exactly what the consequences will be. Such information is not available for any ecosystem. Who will gather more information? Who will fund the work and would the expense involved be justified, given that we still would not know enough?

Second, the greater the potential increase in fish stocks by the introduction of particular species the greater are the potential risks. The more "exotic" the species the more they might be expected to proliferate.

The whole issue of species introductions depends on the question of "which species?" Much more thought is required on this subject and for this reason species are not suggested here. The following might serve as examples only: There could be little objection to transferring archerfishes or engraulids from other rivers in PNG to the Sepik. On the other hand, the introduction of primary freshwater catfishes (e.g., Claridae, Bagridae), despite their probable success in fisheries terms, would not be acceptable because of possible interference with native ariids (Coates 1983).

A slow, cautious and well-planned approach to fish species introductions anywhere in PNG is needed. That approach should ensure maximum safeguards for the native fauna whilst attempting to improve the standard of living of the people in the area. A panel of experienced people would be established to recommend which species might be appropriate and what the dangers might be. That panel would include independent institutions and third parties.

For each species considered, a synopsis of available biological information would be produced and related to known information on the native species. Potential benefits would be weighed against possible risks. After acceptable species are identified, quarantine considerations would be the responsibility of the PNG quarantine services.

A protocol for the evaluation of species introductions would be developed and used for any freshwater organism entering PNG for whatever reason. A possible format has been suggested by Kohner and Stanley (1984) which needs minor alterations for PNG conditions. The procedure would ensure that decisions are based on all possible considerations. Final decisions regarding introductions would rest with the government.

Discussion

A full understanding of the rationale for fish species introductions into the Sepik River system requires familiarity with all data available with an appreciation of the needs of the Sepik people that can only be obtained first-hand. Additional details are available in Coates (in press).

There are few precedents, if any, for these proposals. Fish species introductions into the Sepik River are probably more justifiable than for any other natural water system in the world. More attention and thought has been given to this matter than for any species introduction (fish or otherwise) in Australasia.

It should be noted that the project is a long-term one. Initially it is directed at benefiting the subsistence fishery. In the future, it is hoped that improved stocks will allow commercial fishery to develop. The project is also directed at alleviating the "fish problems" with the river not the "fishing problems". Once suitable stoks were established they would still need to be exploited. Many problems with the development of the fishery would still remain and these would be similar to those encountered in any remote and large tropical river system. But the prerequisite of any fishery is a suitable stock, without which further major developments would be hindered.

A major function of this paper is to solicit help and comment from colleagues in the region who have experience in relevant fields. Comments for or against the project are welcome but all should preferably be supported by as much documentary evidence and data as possible.

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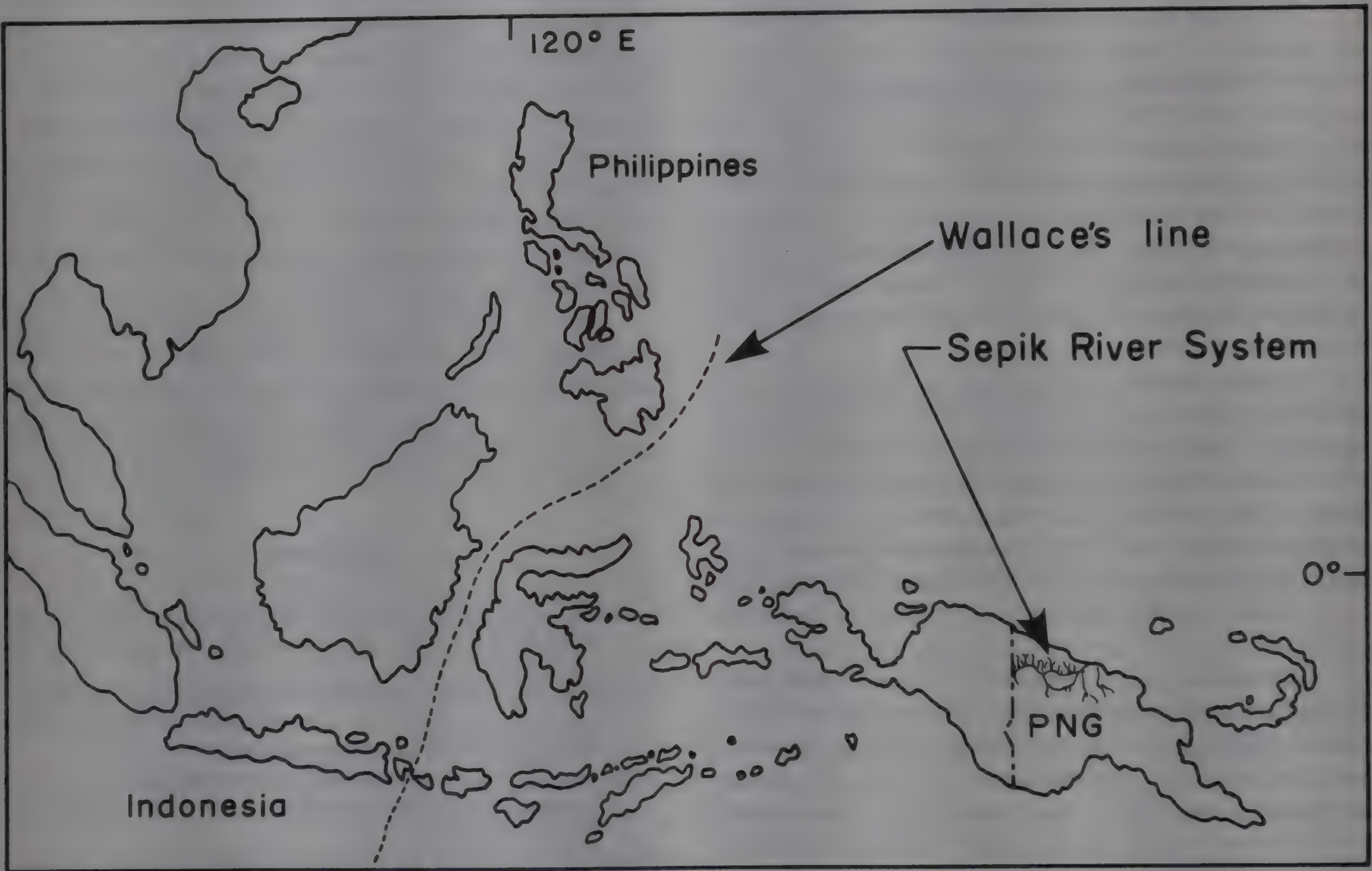
120° E
Philippines
Wallace's line
Sepik River System
0°
PNG
Indonesia

Fig. 1. Southeast Asia and Papua New Guinea, showing the faunal barrier (Wallace's line) and the Sepik River system.

Utilization of Small-Scale Fish Aggregation Devices by Papua New Guinea's Artisanal Fishermen

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Abstract

Fish aggregation devices, commonly known as FADs or payaos, have been one of the most significant introductions to the commercial tuna fishery over the last decade. However, the depth and location at which they are deployed make them too expensive and too far from shore respectively, for exploitation by Papua New Guinea's artisanal fishermen. In this study two FADs were deployed in 160 m and 390 m, respectively, close to the coast (< 10 km). The shallower FAD proved unsuccessful both in the amount of fish it aggregated and the consistency with which they were aggregated. In contrast, the FAD in 390 m provided consistent troll catches averaging 12 kg/hr/vessel with several catches exceeding 40 kg/hr/vessel. The deeper water FAD tripled the annual harvest of tunas by artisanal fishermen in Wewak. *Euthynnus affinis* dominated the species composition around the FAD, while *Thunnus albacares, Auxis thazard, A. rochei* and *Coryphaena hippurus* showed seasonal abundance. *Katsuwonus pelamis, Thunnus obesus* and *Elagatis bipinnulatus* were only caught in small amounts. With the exception of neritic *E. affinis* and *A. thazard*, these species were seldom caught in the artisanal fishery prior to the introduction of FADs. The size range of tunas caught at the FAD (15 to 35 cm caudal fork length) was consistent throughout the year regardless of the species involved. The size ranges were significantly smaller than the size range of fish caught in the artisanal fishery.

Introduction

The main direction of fish aggregating device (FAD) development has been aimed towards the commercial fisheries for skipjack *(Katsuwonus pelamis)* and yellowfin tuna *(Thunnus albacares)*. The main advantage in the use of FADs has been the guarantee of catches and fuel savings due to the shorter searching time. Shomura and Matsumoto (1982) found that the advantages of FADs could be passed onto artisanal and recreational fishermen, who were not only able to increase their catches but were also assured of making daily catches.

Although Mathews and Butcher (1983) and Preston (1982) have shown that catches around FADs could indicate the possibility of commercial troll fisheries, their cost and deployment locations have put them out of reach of artisanal fishermen in many developing countries. Table 1 summarizes the water depths and the distance from shore where the most productive FADs have been deployed as reported by other researchers.

In Papua New Guinea (PNG) the subsistence and artisanal fishermen primarily exploit shallow reef species or troll for neritic pelagic species. In the East Sepik Province of PNG, trolling for mackerel tuna *(Euthynnus affinis)* and frigate tuna *(Auxis thazard)* was the predominant fishing method prior to the recent introduction of deep bottom snapper fishing. Despite the presence of tuna schools throughout the year (Frusher, unpublished data) the expense of searching for schools has decreased the economical success of this method.

To try to increase the harvest of these neritic tunas, the Fisheries Research and Surveys Branch of the Department of Primary Industry began evaluating FADs in shallow water (< 500 m) and close to shore (< 10 km).

Materials and Methods

In April 1984 two FADs were deployed in depths of 160 m and 390 m along the northwestern PNG coastline. The FADs consisted of two or three 200-l foam filled drums welded in an angle iron frame. Twenty-five meters of chain connected the float to the mooring rope and another 25 m of chain connected the rope to the anchor (disused engine blocks). The siting of the FADs made them equally accessible by research station staff and inhabitants from the main fishing villages.

Results

Table 2 gives a list of all the species caught at both FADs, their method of capture and their occurrence.

For the first four months after deployment, 1 x 1 x 0.5 m wire traps were hung from 3-10 m under both FADs. Although these caught a number of small fish, their continued replacement and repair made them impractical and their use was discontinued. These traps mainly caught juvenile trevallies, *Caranx tille* (6-15 cm LCF); finny scad, *Megalaspis cordyla* (8-12 cm LCF); and rainbow runners, *Elagatis bipinnulatus* (18-35 cm LCF) at both FADs.

Vertical longlining was undertaken at both FADs. These longlines consisted of 300 m of mainline (150 m at the 160 m FAD) with hooks clipped on every 15 m. Each branch line consisted of number 12/0 hooks connected to 1 m of trace wire followed by 2 m of 150 kg breaking strain monofilament nylon line. Longlining was only successful at the deeper water FAD where it caught the silky shark, *Carcharhinus falciformes*.

Jigging of baited lines to depths of 35 m caught *E. bipinnulatus* and *C. tille* at both FADs and the dolphinfish, *Coryphaena hippurus*, at the deeper water FAD.

Trolling, using plastic squid or chicken feathers on number 12 hooks attached to 15 kg breaking strain monofilament nylon line, was the dominant method used around the FADs. This was the only method tried which captured tuna.

From July 1984 to July 1985, 8,532 fish were caught around the FADs while only 2,096 fish were caught in the artisanal fishery. The FAD catch comprised mainly fish caught in routine sampling while the artisanal catch represented fish sold to the government fish purchase depot. During this period no other retail outlet was known to have purchased tuna. However, FAD caught tuna was a common sight in the local markets from October to February.

Table 3 gives the species composition of FAD and non-FAD caught pelagic fish. Mackerel tuna dominated both the artisanal and FAD catches, although more so in the artisanal catch. Yellowfin tuna, the second most commonly caught fish at the FADs, was absent from the artisanal catch. The other more oceanic tuna, skipjack tuna, while forming only a small proportion of the FAD catch, was absent from the artisanal fishery. Thus, the FADs gave artisanal fishermen access to a previously unexploited fish resource. In contrast, the more reef-associated pelagic species such as the finny scad, doubled-lined scad, *Grammatorcynus bilineatus* and black kingfish, *Rachycentron canadus* were absent from the FAD catch. Frigate mackerel formed a greater percentage of the artisanal catch and, to a lesser extent, dolphinfish formed a greater percentage of the FAD catch.

Table 4 shows the species composition and catch rate by trolling at the 160-m and the 390-m FADs. The shallow water FAD mainly caught mackerel tuna with minor amounts of frigate and skipjack tuna, rainbow runner and dolphinfish. While mackerel tuna was the dominant species at the offshore FAD, yellowfin and frigate tunas were also caught in considerable quantities. The lack of yellowfin, and to a lesser extent, skipjack tuna at the shallow water FAD indicates the more oceanic preferences of these tunas.

Fig. 1 shows the monthly CPUE data from the first catches of fish at the deeper water FAD in July 1984 to September 1985. From these data it can be seen that after a period of increased catches from August 1984 to March 1985 there has been a gradual decrease in CPUE figures. Whether this is due to the FAD losing its effectiveness as an aggregator or seasonal differences is presently unknown.

Table 4 shows the mean CPUE of each species caught at the shallow and deepwater FADs. The deeper water FAD caught approximately seven times the number of fish per hour than did the shallow water FAD. All species caught showed higher catch rates at the deeper water FAD.

Mackerel tuna was the dominant fish caught at the FAD. Yellowfin tuna was the second most common fish caught and appeared to be seasonal from July/August to February. Frigate tuna were slow to start aggregating at the FAD and after March they became more important, dominating the FAD catch in September 1985. Of interest is the first positive identification of the wide corsetted frigate tuna *A. rochei* in September 1985, when it formed 47.1% of the frigate tuna catch. The presence of *A. rochei* may be related to a seasonal parameter and may be responsible for the increased frigate tuna catches from March 1985. Skipjack tuna appeared in only minor quantities and was most abundant from July/August to December. Dolphinfish had a definite seasonal period of abundance at the FAD from November till April, while rainbow runners appeared as irregular visitors to the FAD from September to May.

For all species, the FAD-caught fish were significantly ($p < 0.01$, t-test) smaller than fish caught in the artisanal catch. Only in the dolphinfish and the frigate tuna did the upper limit of the size range caught at the FAD include maturing fish. In all four tuna species the size range caught at the FAD was similar, irrespective of the maximum size of the species, its size at first maturity, or whether neritic or oceanic. Even though the shallow water FAD was placed in a region where adult mackerel tuna have been caught, only juveniles were caught in the vicinity of the FAD, although adults were still caught in the same region. Thus FADs deployed in depths of up to 390 m in northwestern PNG aggregated a select size range of fish, mainly juveniles.

Discussion

While FADs appear as a bonus to PNG's artisanal fishermen their harvest of juvenile fish is of concern. In Fiji, Preston (1982) found a nearshore FAD often to have 'very small tuna'. At the deeper water FAD, trolled skipjack tuna averaged 2.4 kg and 10% of the trolled yellowfin tuna were greater than 10 kg. Floyd and Pauly (1984) consider the reduction in the commercial Philippine tuna catch to be associated with growth overfishing by using FADs.

Kihara (1981) reports that juvenile yellowfin and skipjack tunas were seasonally caught by purse seiners at FADs in the Philippines, primarily during May and June. In Fiji, Preston (1982) found that the purse seine catches around FADs yielded slightly smaller skipjack tuna than the free-ranging schools fished by the pole and line vessels. While purse seining is a less selective method than pole and line fishing, the available data indicate that FADs aggregate tunas from juveniles to adults whereas free-ranging schools appear to be composed of larger adult fishes. Thus the harvesting of tunas at FADs may remove a built in safety margin that prevents growth overfishing.

Of importance to the artisanal fishermen is the increased harvest of juvenile mackerel and frigate tunas. These tunas also support artisanal troll fisheries. Little work is available on the extent of migrations and population structure of these species. Work on other scombrids has shown that neritic species may have more defined population structures than the oceanic species. Shacklee (pers. comm.) used electrophoretic techniques to demonstrate genetic differences in *Scomberomorus commerson* between PNG islands region (Kavieng) and the northern PNG mainland (Wewak and Tufi) as well as between eastern and western Australia. Wilson (1981) using meristic, morphometric and electrophoretic evidence suggests that the neritic longtail tuna *(Thunnus tonggol)* has a PNG (samples from Port Moresby and Gulf of Papua) as well as a western population (western Australia, Malaysia). If mackerel or frigate tunas form discrete localized populations then artisanal exploitation of juveniles at FADs may have a deleterious effect.

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Table 1. Summary of water depths and distance from shore where researchers have found their FADs most productive.

Researchers	Country	Distance from coast (km)	Water depth (m)
Matsumoto et al. (1981)	Hawaii	not mentioned	2,000
Kihara (1981)	Philippines	> 50	> 3,000
Brock (cited PTDF 1979)	Hawaii	not mentioned	> 1,600
Preston (1982)	Fiji	40	not mentioned
de San (1982)	Western Samoa	20	1,800

Table 2 Species list of fish caught at shallow water (160 m) and deepwater (390) FADS and their capture method and occurrence.

Species	160-m FAD		390-m FAD	
	Capture method	Occurrence	Capture method	Occurrence
Aluterus monoceros	T	R	T	R
Aluterus scriptus	T	R	T	R
Decapterus russelli	—	—	T	R
Megalaspis cordyla	T	R	T	R
Selar cremnopthalmus	T	R	T	R
Seriola rivulana	—	—	TL	R
Elagatis bipinnulatus	T, TL, J	R	T, TL, J	M
Acanthocybium solandri	TL	R	—	—
Sphyraena barracuda	—	—	TL, J	R
Caranx tille	T, J	M	T, TL, J	M
Psenes cyanophrys	—	—	T	R
Lobotes surinamensis	T	R	—	—
Carcharhinus falciformes	—	—	LL	M
Thunnus obesus	—	—	TL	R
Thunnus albacares	TL	R	TL	C
Katsuwonus pelamis	—	—	TL	M
Auxis rochei	—	—	TL	M
Auxis thazard	TL	R	TL	M
Euthynnus affinis	TL	M	TL	C
Coryphaena hippurus	TL	R	TL, J	M

T = Trapping, TL = Trolling, J = Jigging and LL = Vertical longlining.

R = Rare: either <20 individuals caught during the survey or >20 individuals caught on 1 or 2 occasions.

M = Medium: > 20 and < 500 individuals caught during the survey.

C = Common: >500 individuals caught during the survey.

Table 3. Species composition of FAD and nonFAD trolled pelagic fish from July 1984 to September 1985.

Species	FAD		NonFAD	
	No.	%	No.	%
Euthynnus affinis	4,871	57.1	1,503	71.7
Auxis thazard	1,009	11.8	371	17.7
Thunnus albacares	2,122	24.9	0	0
Katsuwonus pelamis	223	2.6	0	0
Coryphaena hippurus	188	2.2	8	0.4
Elagatis bipinnulatus	119	1.4	65	3.1
Megalaspis cordyla	0	0	112	5.3
Rachycentron canadus	0	0	16	0.8
Grammatorcynus bilineatus	0	0	16	0.8

Table 4. Species composition and catch rate (number of fish/hour) by trolling at the 160-m FAD and the 390-m FAD from July 1984 to May 1985

Species	160-m FAD			390-m FAD		
	No.	%	No./hr	No.	%	No./hr
Euthynnus affinis	72	80.0	4.06	4,637	56.4	20.85
Auxis thazard	4	4.4	0.23	937	11.4	4.21
Katsuwonus pelamis	6	6.7	0.34	222	2.7	1.00
Thunnus albacares	0	—	—	2,116	25.8	9.51
Elagatis bipinnulatus	4	4.4	0.23	119	1.4	0.54
Coryphaena hippurus	4	4.4	0.23	186	2.3	0.84

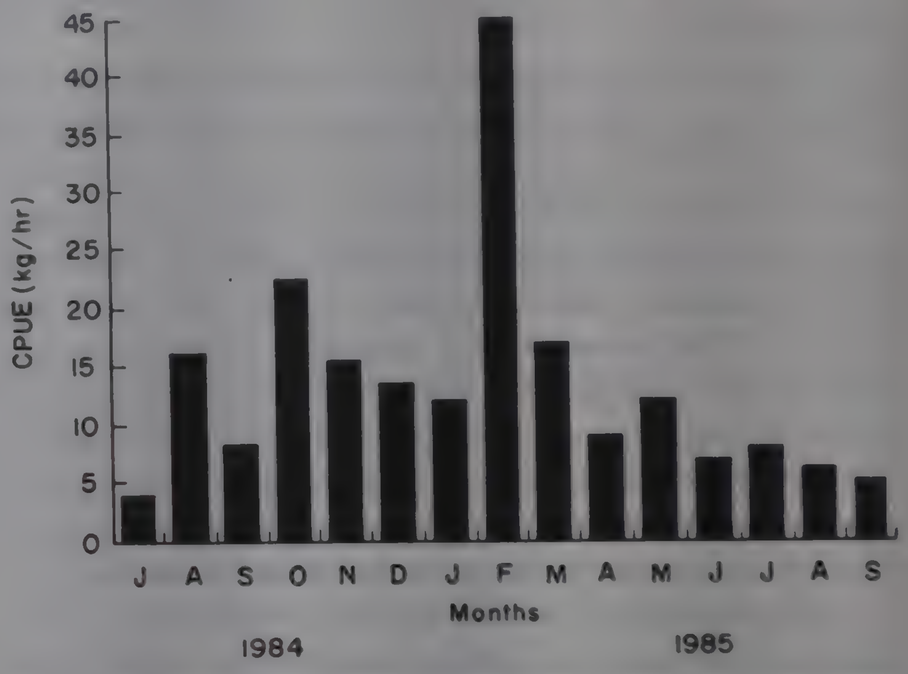

Fig. 1. Monthly CPUE data for the 390-m FAD from July 1984 to September 1985.

The Development and Management of the Northern Territory Barramundi *(Lates calcarifer)* Fishery

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Abstract

Barramundi *(Lates calcarifer* Bloch*)* is a large catadromous centropomid fish exploited by recreational and commercial fishermen in rivers and estuaries of northern Australia. The Northern Territory commercial fishery for barramundi developed rapidly during the 1950s. Management regulations have been progressively introduced for the commercial fishery since 1940. Reviews of the status of the resource, based on surplus production models, have been undertaken since 1975. These have resulted in more stringent regulations including license limitation, seasonal and area closures, gear restrictions, and more recently a license buy-back scheme. Since the 1970s, the extent of exploitation by recreational fishermen has increased significantly along with population growth and development of previously remote areas. Also, an increasing awareness of the economic benefits of recreational fishing, particularly through tourism, has resulted in the active promotion of barramundi fishing as a tourist attraction. Although there is a high demand for the commercial supply of barramundi to the consumer, there is a growing demand for recreational utilization of the same resource. Consumer demand for barramundi may eventually be satisfied by aquaculture.

Introduction

Commercial exploitation of barramundi or seabass *(Lates calcarifer* Bloch*)* began in the Northern Territory soon after the establishment of the permanent settlement at Port Darwin, but it was not until the advent of postwar technology in the 1950s that the fishery provided for more than local market sales.

The commercial fishery expanded rapidly from 1960 to a peak in 1976-1977. This increase in total catch and number of fishermen and a decline in catch rate prompted the first of a series of reviews of the fishery from 1978. Stock assessment analysis based on the Schaefer surplus production model demonstrated that the resource was subject to excessive commercial fishing effort. Management measures to address this problem were introduced progressively from 1978. Since then the rate of population growth in the Northern Territory has increased significantly with a concomitant increase in recreational fishing. With the perceived need for economic diversification and development of the Northern Territory, the Government has encouraged tourism including the promotion of barramundi angling.

Because of the increase in recreational fishing there has been support within the community for the need to 'allocate' proportions of the resource to both user groups. While to date this proportioning has been achieved by regulation of the commercial fishery for its own sake, increasing pressure from recreational fishermen could result in the introduction of measures to achieve formal resource allocation by area, and/or catch quota or other means.

This would require detailed assessment of the recreational fishery, more reliable estimates of yield, a better understanding of the biology and life history of the barramundi and consideration of a wide range of other issues which have bearing on the management of the fishery (e.g., environmental fluctuations).

This paper briefly outlines the biology and life history of barramundi; the commercial and recreational fishery; stock assessment; and future management of the fishery.

Biology and Life History of *Lates calcarifer*

Barramundi is a large centropomid fish, widely distributed in rivers and estuaries in tropical and semi-tropical regions of the Indo-West Pacific (Greenwood 1976). Studies on this species in northern Australia and Papua New Guinea have identified that it has a complex life history, being a protandrous hermaphrodite and catadromous (Moore 1979, 1980, 1982; Moore and Reynolds 1982; Reynolds and Moore 1982; Davis 1982, 1985; Russell and Garrett 1983, 1985; Griffin, in press).

In the Northern Territory, spawning occurs in August or September until February in brackishwaters (28-36 ppt) near the mouths of rivers. Movement to the spawning areas and maturation of gonads is thought to be triggered by an increase in water temperature which occurs at the end of the dry season (southeast monsoon). There is a tidal based monthly cycle of spawning, with postlarvae entering coastal swamps on the peak spring tides. As the wet season (northwest monsoon) develops, these swamps and adjacent flood plains back up with freshwater and the young barramundi remain in this safe, productive environment until flood waters recede around March or April (Davis 1985).

At this time, the young of the year fish move upstream to the upper reaches of the river where they generally remain until they are 3 to 4 years of age (60-70 cm total length or 2.6-4.2 kg). At this time they reach sexual maturity as males and at the end of the next dry season will migrate downstream to spawn depending on access to tidal waters (Fig. 1).

These fish remain in the tidal waters of the river, and each male will participate in spawning several times before changing sex at between 6 and 8 years to become female (protandry). At this age, the fish are 85 to 100 cm in length and between 7 and 12 kg (Davis 1982).

Female barramundi have a high reproductive capacity, producing up to 10 million eggs at 100 cm and 30-40 million at 120 cm (Davis 1984).

This movement, and subsequent residency of older fish in tidal waters creates an age aggregated population, i.e., young immature fish up to 4 years of age tend to be found upstream and in freshwaters while the older larger fish are found predominantly in tidal and estuarine waters.

Commercial Exploitation

In the Northern Territory and the rest of northern Australia, barramundi are captured for commercial purposes by gill netting. Most fishermen use monofilament or multifilament gill nets of 150 mm or 180 mm mesh size. These nets are set in rivers, estuaries and along the coast, in lengths of 25 to 1,000 m (and in previous years up to 4,000 m) depending on the locality.

Fishermen generally base their operations in a particular river system and their vessels provide a work platform and have freezer capacity sufficient to hold catches taken over extended fishing periods.

The size composition of commercially caught fish is generally within the range of 50 to 140 cm total length, with a mean between 66 and 85 cm (3.5-7.1 kg) depending on the river system (Rohan et al. 1981).

Development of the commercial fishery began in the late 1930s but significant expansion of the fishery occurred from 1961 onwards, the catch increasing from less than 10 t liveweight in 1953-1954 to approximately 118 t in 1959-1960 and to a peak of 1,162 t in 1976-1977 (Fig. 3).

Recreational Exploitation

In Australia, recreational fishing is a very popular pasttime and the expansion of the recreational fishery for barramundi in the Northern Territory is being facilitated by the development of tourist infrastructure, fishing charter and safari operations and a vigorous tourist promotion program both within Australia and overseas.

Recreational fishing for barramundi is predominantly from small outboard powered dinghys. The fishermen use rods and light monofilament line with lures or bait, although the casting and or trolling of lures are by far the most popular methods. The majority of recreational fishing takes place in the freshwaters of the major river systems and their tributaries or billabongs (seasonally isolated waterholes).

The heaviest amateur fishing pressure is applied in rivers which are readily accessible from Darwin, e.g., the Alligator and Mary River systems. Other popular areas are the Daly, Finnis, Roper, Victoria and MacArthur Rivers (Fig. 2).

A survey in 1978-1979 assessed the extent and success of amateur fishing in the Mary and Alligator River systems (Griffin 1982) and indicated that amateurs caught approximately 80 t of barramundi during the 12-month survey. A total of 2,205 anglers were interviewed; total catch was 3,602 with an average estimated weight of 3.1 kg.

Expenditure on equipment and related costs by amateurs fishing in that area was approximately A$1.9 million. It was estimated that amateur fishermen captured 10-20% of barramundi landed in the Northern Territory. These figures also suggested that the annual value of recreational barramundi fishing was of the same magnitude as the commercial catch (Griffin 1982). A survey of seafood consumption in Darwin in 1983 revealed that over 45% of consumers engage in fishing for recreation and that 64% of households regarded barramundi as the most popular species taken (Bandaranaike, unpublished data).

Stock Assessment

In view of the lack of estimates of population parameters, the assessment of yield from the barramundi fishery has been based on the surplus production model (Schaefer 1954). Recently, modified surplus production models have been developed including that of Fox (1970, 1975), and recent yield assessments for the fishery were undertaken using both methods.

Initially, assessments of the state of the barramundi population were made with data from the whole of the Northern Territory, i.e., treating it as one stock. This approach was useful for determining theoretical total effort levels for the fishery as a whole. However, as there is substantial evidence that there are a number of stocks based on individual river systems (Shaklee and Salini 1985), estimates have been made of yields and optimum effort for individual river systems as well as for the total fishery. The reliability of these estimates varies, and these estimates are only used as a broad indication of the status of the fishery.

Data on catch and effort are collected from commercial fishermen through individual monthly returns and include catch by species, location of fishing operations, length of net used, mesh size and the number of days fished. Effort is measured in 100-m net day (HMD), a simple product of the length of net used and the number of days that it is set.

Catch and effort data for the Northern Territory commercial fishery and the Mary River system are provided in Table 1. Estimates of yield only apply to the commercial sector of the fishery and it is not possible to provide an expected yield for the whole fishery with any level of confidence.

While the level of recreational catch is low compared to the commercial catch it can be treated as part of the natural mortality component. However, in the last decade the level of recreational fishing has increased substantially in some areas and studies are being initiated to provide additional information to enable the incorporation of recreational catch and effort into a total yield assessment.

Management of the Fishery

The first specific management regulation for the barramundi fishery in the Northern Territory was issued in 1962. This closed inland waters to commercial gill net fishing on a seasonal basis in the mistaken belief that barramundi spawned in freshwater. This was further extended to a total closure in 1966 and other regulations including mesh size limitations and a minimum legal length (58 cm) were imposed.

In 1969, further regulatory measures were introduced, based on results of limited biological research undertaken in other parts of Australia.

An assessment of the economic status of the commercial barramundi fishery was carried out in 1975 as part of an overall review of the Northern Territory fishing industry. A major recommendation was that the number of licenses issued for the barramundi fishery should be limited, based on economic criteria (Copes, unpublished report). Limitation was introduced in 1976, and at this time the number of licenses was set at 170 but subsequently reduced to 154 in 1977.

License limitation did not prove effective in controlling the fishing effort which increased rapidly from 15,741 HMD in 1975 to over 90,000 HMD in 1978 and 1979. As effort increased, a marked reduction in catch rate occurred resulting in industry concern. In 1978 a full review of the fishery was undertaken (Grey and Griffin 1979), and the Schaefer surplus production model was applied to the catch and effort data for the period 1972-1978. This analysis provided estimates of maximum sustainable yield (MSY) and optimum fishing effort (E), of approximately 1,000 t and 70,000 HMD, respectively. As the effort applied in 1978 exceeded this theoretical optimum, several new measures were introduced to limit fishing effort. Although it was recognized that the yield estimates were of limited reliability, these measures were imposed in the light of the potential increase in effort (through longer net lengths and increased fishing time). A maximum net length of 1,500 m was imposed and the three-month closed season (introduced in 1977) was extended to four months from 1 October to 31 January.

Since 1979 the management of the fishery has been under constant review. Rohan et al. (1981) included 1979 and 1980 data and gave a revised estimate of MSY of about 890 t and E of 61,500 HMD.

In 1982-1983 management concentrated on reducing fishing effort by reduction of net length to a maximum of 1,000 m (some were allowed up to 1,500 m) and reduction of the number of fishermen through a license buy-back scheme. Other measures were introduced to reduce potential and actual effort including increases in buy-back price and endorsement fees.

A review of the fishery in 1985 indicated that although the license buy-back scheme had been successful in reducing the number of licenses there was still a need for further reduction (Grey and Griffin, unpublished report). This review included data from 1972 to 1984 inclusive (Table 1). The Schaefer estimated MSY was 886 t for 59,900 HMD effort, whereas the Fox estimate for MSY was 771 t for 61,300 HMD (Fig. 4).

In addition, this review recognized the increasing impact on the resource by the recreational fishermen and additional measures were imposed to provide restraint on commercial fishing activities in the Mary River system in particular, as well as imposing a further reduction on the fishery in general through reduction in total net lengths permitted.

In January 1986, 41 licensed fishermen were endorsed to operate in the barramundi fishery.

Although the majority of the management measures introduced to date relate specifically to the activities of commercial fishermen, recreational fishermen are not permitted to sell their catch and a bag limit of 5 fish per person per day with a maximum of 10 fish in possession was introduced in 1980.

Future Considerations

In recent years the question of the allocation of the barramundi resource to the recreational and commercial fishermen has been subject of considerable debate. There is no doubt that the requirement by recreational fishermen for the resource will further increase with population, accessibility and increasing leisure time. At the same time, the demand for commercial product for the tourist consumer and the higher national demand for the species will require maintenance of the commercial fishery at a sufficient level to satisfy these demands.

The demand for access to the resource has become a political issue to some extent, with both groups pressuring the Government for increased consideration. Recreational fishermen have put forward proposals to close specific rivers to commercial fishing. These proposals have been made on the premise that commercial fishing is having a detrimental effect on stocks and its removal would result in increased expectations of catches by amateur fishermen. There is no doubt that the groups are both competing for the same resource. This competition is likely to further develop into conflict unless the appropriate management measures are introduced, or the availability of the resource is increased.

To date there have been a limited number of unsuccessful attempts to culture barramundi in the Northern Territory, although recently some progress has been made on aquaculture of this species elsewhere in Australia (Young 1985; Anon. 1986). When the aquaculture production of barramundi reaches the point of commercial production, this will alleviate to some extent the commercial demand for the species to be taken from the wild stocks.

Until this is achieved, the management of the fishery will require the careful adjustment of the exploitation levels by both groups in order to achieve the appropriate balance and maintain the long-term viability of the wild stock population. To this end an ongoing program of commercial and recreational fishery assessment must continue and monitoring of the status of the resource maintained.

Acknowledgements

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Table 1. NT barramundi fishery catch and effort data, 1972-1984.

Northern Territory			Mary River		
Year	Catch (t)	Effort (HMD x 1,000)	Year	Catch (t)	Effort (HMD x 1,000)
1972	382.0	17.3	1972	178.5	8.8
1973	431.3	21.0	1973	155.2	9.1
1974	656.0	22.8	1974	239.1	9.5
1975	432.3	15.7	1975	141.7	7.2
1976	973.8	–	1976	–	–
1977	1,054.0	72.0	1977	270.2	22.0
1978	820.0	95.9	1978	152.8	22.8
1979	745.0	100.7	1979	137.5	18.5
1980	531.7	71.4	1980	137.2	19.6
1981	764.1	66.9	1981	191.1	16.0
1982	856.1	95.4	1982	170.5	24.9
1983	603.1	87.1	1983	72.2	17.7
1984	617.1	71.6	1984	126.6	19.3

N.B. 1976 Data not available.

	Model Parameters			Model Parameters	
	Schaefer	Fox		Schaefer	Fox
r^2	0.85	0.85	r^2	0.70	0.62
E (HMD)	59,900	61,300	E (HMD)	13,500	10,630
MSY (t)	886	771	MSY (t)	205.3	188.2

HMD = hundred-meter net day

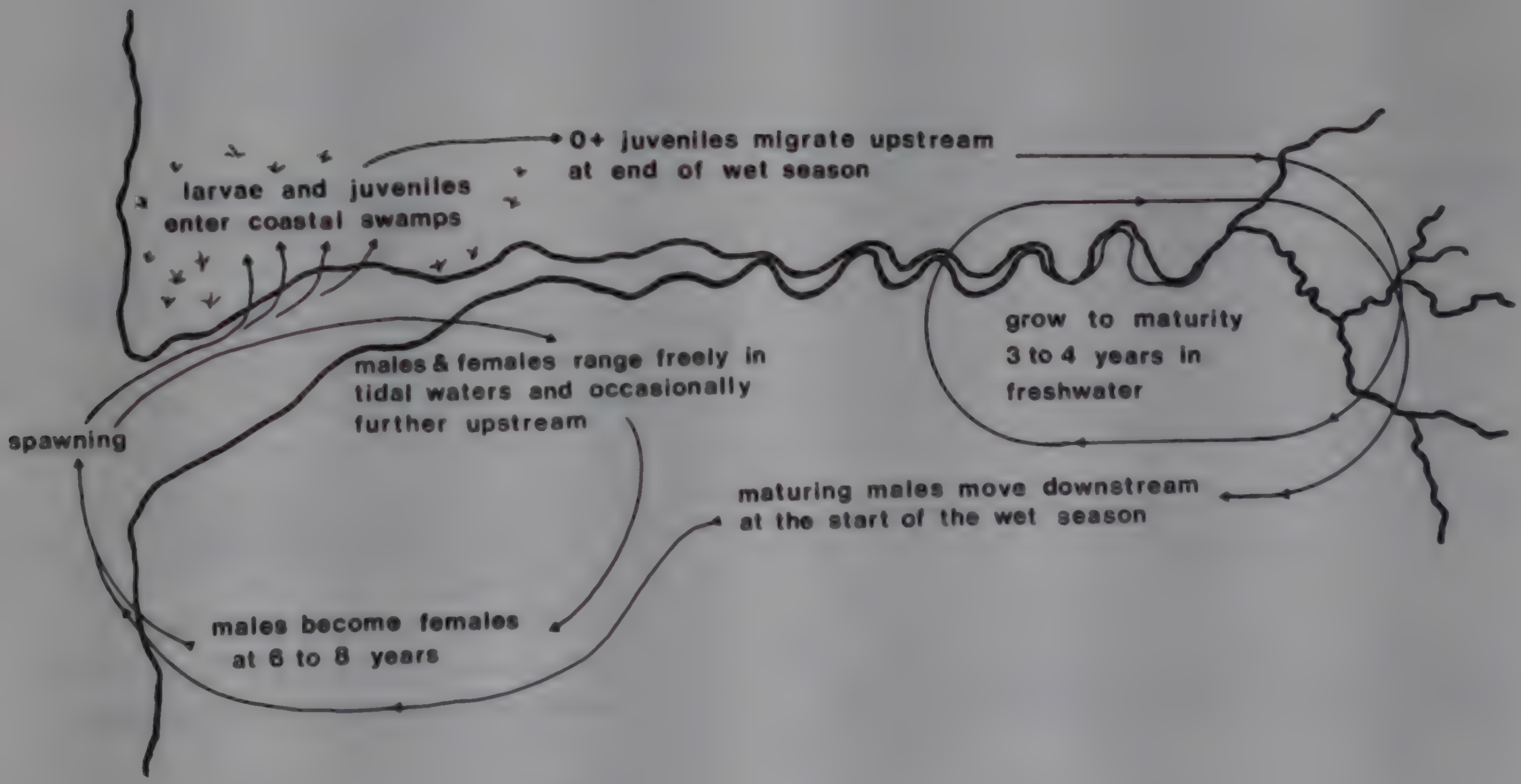
0+ juveniles migrate upstream at end of wet season
larvae and juveniles enter coastal swamps
grow to maturity 3 to 4 years in freshwater
males & females range freely in tidal waters and occasionally further upstream
spawning
maturing males move downstream at the start of the wet season
males become females at 6 to 8 years

Fig. 1. Generalized life history of barramundi *(Lates calcarifer)*.

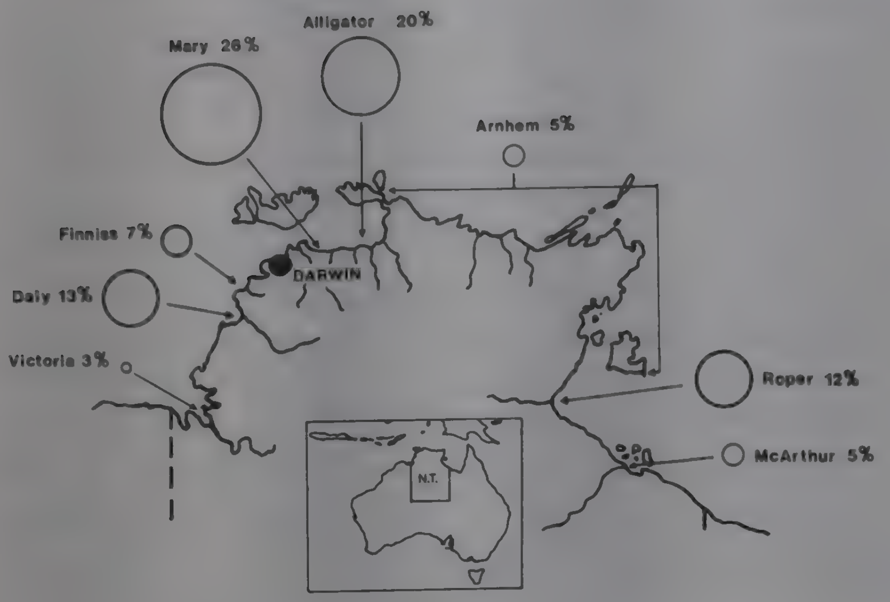
Alligator 20%
Mary 26%
Arnhem 5%
Finniss 7%
Daly 13%
DARWIN
Victoria 3%
Roper 12%
McArthur 5%
N.T.

Fig. 2. N.T. barramundi fishery landings by river system (mean 1972-1984).

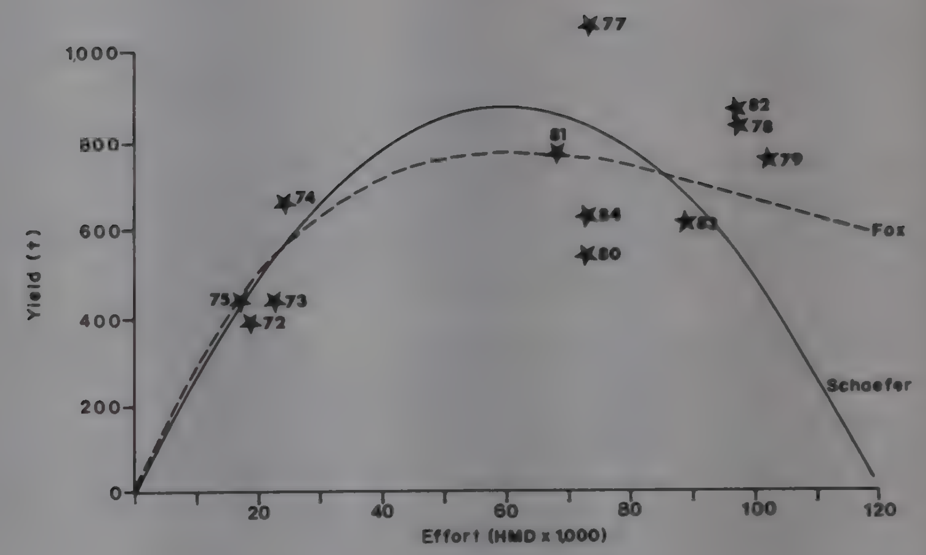

Fig. 4. Schaefer and Fox yield curves—total *Lates calcarifer* fishery. HMD = Hundred-meter net day.

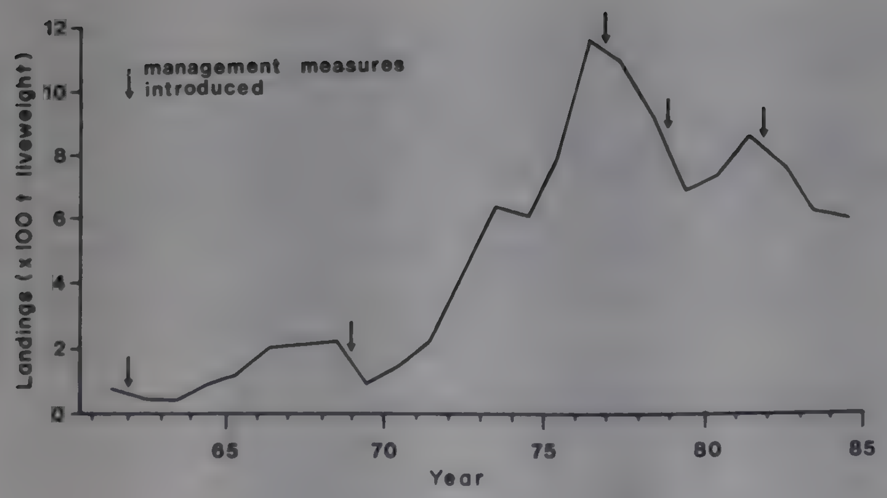

Fig. 3. N.T. barramundi landings 1961/1962-1984/1985.

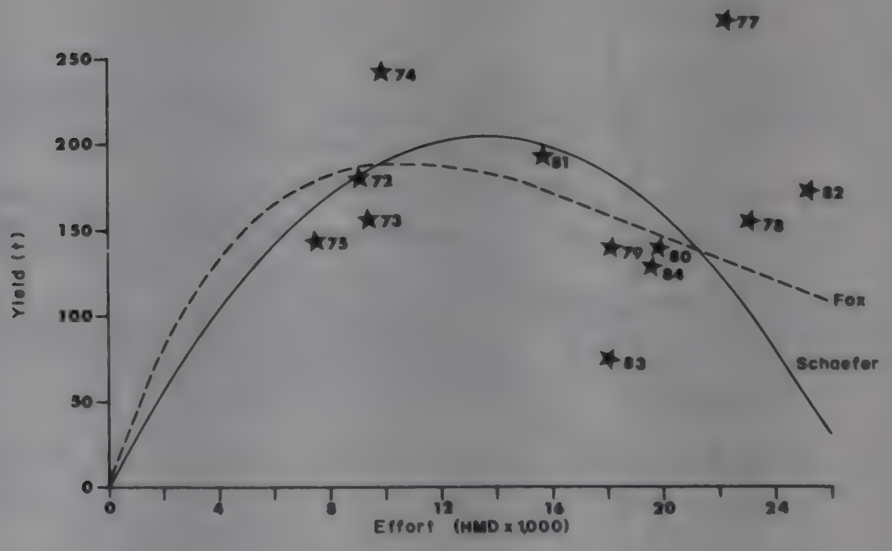

Fig. 5. Schaefer and Fox yield curves—Mary River.

Spiny Lobster Fishery in Eastern Samar, Philippines

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Abstract

Thirteen stations throughout eastern Samar, Philippines, where specific groups of lobster fishermen operate, were monitored to derive baseline information about the lobster resources in the area including an overview of the marketing aspects involved.

Six types of fishing gear were used for capturing lobsters: gill nets, fish traps, fish corrals, hook and line, local spear guns and lobster traps. Catches from the first four are mainly incidental while a deliberate spear and trap fishery exists. The peak lobster season generally occurs from May to August, coinciding with the calmest periods on the Pacific coast.

Six species and subspecies were recorded from the area: *Panulirus penicillatus, P. ornatus, P. longipes longipes, P. longipes femoristriga, P. versicolor* and *P. homarus homarus*. Of these, *P. penicillatus* which abounds in the surf areas on the Pacific coast, comprises the bulk of the landed catch. A general decline in catch per unit effort expressed as total catch (lobster tails in kg)/fisherman/fishing trip was noted during the peak lobster seasons in 1982 to 1984.

Introduction

Spiny lobsters are among the most expensive marine commodities with a high demand in both local and international markets. The total reported volume of harvested spiny lobsters from the Philippines amounted to as much as 1,457 t in 1979 but declined to 965 t in 1982. Total exports of frozen or chilled lobster tails amounted to 66,388 kg in 1982 with an estimated value of ₱3,470,785.00 (BFAR 1982). However, little is known about the local spiny lobsters, much less their fishery. In 1983, the University of the Philippines Marine Science Institute initiated a lobster research project to derive baseline information on aspects of the biology and ecology of local species *(Panulirus* spp.*)*. Guiuan, eastern Samar, one of the regular buying stations for exported lobsters, was chosen as one of the study areas for the local lobster fishery.

Methods

The daily landed catches at the buying stations were monitored regularly from November 1982 to November 1983. As much as possible, the number of lobsters per species was noted from the available catch. Extensive interviews were conducted with fishermen in various localities throughout eastern Samar to derive information on the state of the fishery. In addition, questionnaires were sent out to gather information regarding the species caught, gear used and seasonality of the fishery including some aspects of marketing.

Estimates of catch per unit effort (CPUE), expressed as the total catch (kg)/fisherman/successful fishing trip, were derived from the daily records of catches of fishermen in selected stations. Successful fishing trips refer to trips when lobsters were caught. The individual average CPUEs were derived by dividing the total monthly catch of each fisherman by the number of successful fishing trips. The average monthly CPUE represents the mean of these individual CPUE for each month. The relative abundance of the various species was based on their frequency in the landed catch.

The areas surveyed in eastern Samar extended from Dolores in the north down to Homonhon Island. Thirteen stations delineating general areas where specific groups of lobster fishermen operated regularly were identified. The general types of fishing areas were: rocky surf areas along the Pacific coast, coral reef areas and sandy muddy areas with a poorly-developed coral community in shallower portions. The thirteen stations and the respective general type(s) of lobster fishing grounds are indicated in Fig. 1.

Results

Six species and subspecies were recorded: *P. penicillatus (kawakaw, badason); P. longipes longipes (buranting); P. longipes femoristriga (buranting); P. ornatus (tigbi-on); P. versicolor (dara-atan);* and *P. homarus homarus (badason)*. The distribution and relative abundance of each species in the various stations were related to the available habitats in the area (Fig. 1). *P. penicillatus* abounds in the rocky surf zones where there is high wave action. Consequently, it is the most common species caught in the exposed areas along the Pacific coast (Stations 1-3). Both subspecies of *P. longipes* as well as *P. versicolor* thrive in coral reef areas with clear and calmer waters (Stations 4b and 11). The latter species, however, is also caught in more turbid waters with muddy sandy substrates where *P. ornatus* is commonly caught (Stations 5b, 12 and 13). *P. homarus homarus* is the rarest. Fishermen generally do not distinguish this as a distinct species and it is thought to be the female *P. penicillatus*. It was reported to be most commonly caught also in the surf areas.

The overall species composition of lobsters landed in Guiuan can be seen in the monthly frequencies per species in the sampled landed catch from November 1982 to November 1983 (Table 1). *P. penicillatus* is clearly the most dominant species in the area comprising over 80% of the landed catch. *P. longipes* (both subspecies pooled) is the second most common species (10.8%) followed by *P. versicolor* (4.7%) and *P. ornatus* (1.8%). The number of *P. homarus homarus* in the landed catch is negligible compared to the other species.

Six types of fishing gear were found to be used for capturing lobsters: fish traps, fish corrals, hook and line, gill nets, local spear guns and lobster traps. Catches with the first four types of gear are incidental. It was however, reported that gill nets are used in Station 1 to deliberately catch lobsters during stormy times of the year when the northeast monsoon is prevalent. The nets are set along the edges of the surf zone in the afternoon and retrieved early in the morning the next day. The lobsters are thought to come out of their shelters to feed on organism caught in the nets and in the process get entangled themselves. On the other hand, a deliberate spear and trap fishery (discussed below) based primarily on *P. penicillatus* exists in the area during the "peak lobster season" which coincides with the calmest time of the year. This season generally extends from April to August when most of the stations are protected from the prevailing southwest monsoon.

All species are caught by spear fishermen. Only *P. penicillatus*, followed by *P. longipes*, are commonly caught in lobster traps although *P. versicolor* was also reported to be rarely caught in traps in Station 6. The first is also the most common species caught by gill nets. *P. versicolor* and *P. ornatus* were reported to be ocassionally caught in fish traps and fish corrals and by hook and line.

The gear of spearfishermen consists of improvised wooden spear guns, a flashlight sealed in strips of tire inner tubes and rubberbands, wooden fins and goggles. Activities of these fishermen are concentrated at depths of 4-8 m in surf and coral reef areas. A few of the spearfishermen in Stations 1 and 2 used hookah with breathing hoses to catch lobsters at depths of 12-50 m in more protected areas (embayments in Stations 1 and 2). In addition to the abovementioned gears, divers use heavy chains as weight belts and an improvised mask with a plastic helmet to which a waterproof flashlight is attached at the center of the forehead (similar to a miner's helmet). While these spearfishermen go primarily for lobsters during the peak season when the prices of lobsters are highest, they also spear fish.

Fishing effort varies with the physical capacity of the fishermen; however, most deliberate lobster fishermen go out at least 4-6 days a week during the "lobster season." They often dive for as much as 6 hours per night in two shifts from 9 p.m. to 12 midnight and from 2 to 5 a.m.

A small-scale trap fishery for *P. penicillatus* exists in Stations 3 to 6. In each station, there are 5-10 fishermen with about 6-8 traps each. Cylindrical bamboo traps with openings at both ends which are lined with bamboo slats are used. These are baited with crushed sea urchins *(Echinometra* sp.) and set out in surge channels and undercuts in the surf zone secured in place by wooden stakes during low tide and retrieved the next day.

While traps can be used throughout the lobster season when the Pacific coast is calm, they are used mainly only from March to May. As the activities of spearfishermen intensify, most trap fishermen stop their operations inasmuch as the former reportedly loot the catch at night and destroy the traps to discourage competition. Some in fact shift to spearfishing for the rest of the season.

Of the thirteen stations, sizeable data were available only from Stations 6 and 7 as most spearfishermen from these stations take their daily catch directly to the buying stations where records were obtained. Only relatively complete records of some fishermen were used. Estimates of average CPUE for May-August (1982-1984) in these stations are shown in Tables 2 and 3. Over these years, there was a decline of about 100 g (lobster tails) in the average daily catch of spear fishermen in the area.

For spearfishing with compressor, the only data available were from Station 1 in May 1984. Two teams, each composed of two divers, had an average daily catch of 5.3 kg lobster tails (S.D. = 1.46) mainly large *P. ornatus*. On the other hand, average daily total catch (whole lobsters) from 6-8 lobster traps of one fisherman was only .20 kg (S.D. = 0.63).

Table 4 shows the total monthly shipments of lobsters from Guiuan, eastern Samar from 1973 to 1984 based on Bureau of Fisheries and Aquatic Resources (BFAR) auxillary invoice reports. It should be noted that most of the shipments are lobster tails rather than whole lobsters. Based on yearly totals, shipments for export reached its peak in 1976, declining drastically in 1979 to 1981 and picking up again thereafter.

Lobsters caught from various stations are brought to Guiuan where four exporting firms have buying stations. In stations far from Guiuan, spearfishermen sell lobsters to local brokers who bring them to Guiuan every 2-3 days. Spearfishermen from nearby stations (Stations 5-7 and 11), on the other hand, bring their catch directly to the buying stations to get the best price. Usually, only tails are bought. Degutting and headpopping are done by the fishermen themselves. However, from March to August, whole uninjured lobsters caught by traps are bought by exporters.

Lobster tails are sorted and priced according to size regardless of the species. During the "lobster season", prices increase due to competition between the buyers who try to outprice each other to get the largest lobster supply. In 1984, regular tails (90-300 g) sold from ₱75 to as much as ₱150/kg. Oversized and undersized tails sold for half the price. Whole lobsters (150-400 g), on the other hand, sold for ₱30-60/kilo. Prior to transport to Manila either by ship or plane from Tacloban, lobsters are thoroughly cleaned and packed in crushed ice to a lobster weight ratio of 1:1. Final processing, blast freezing and final packing for export are undertaken in export processing plants in Manila.

Quoted export prices as of May 1984 ranged from $3.75 for 453 g (1 lb) of 28-57 g (1-2 oz) lobster tails to $6.00/lb for lobster tails > 300 g/piece. Prime cuts (100-300 g/piece) fetched up to $14.85/kg. They were reportedly sold in outlets in the United States for about $26.00/kg.

Discussion

The general distribution of the species in relation to their typical habitats largely conforms with the observations of other workers (de Bruin 1969; Pyne 1970; Berry 1971; George 1974 and Bhatia 1974). In general, *P. penicillatus* has the lowest tolerance for turbid waters; thus it abounds in the surf zone where the waters are clear. *P. homarus homarus* favors a similar habitat. Both subspecies of *P. longipes* are associated with living coral reef areas where there is low to moderate turbulence and turbidity. *P. ornatus* lives in highly turbid waters up to depths of 50 m. *P. versicolor* is found in the widest range of habitats (turbid waters, surf areas, coral reef areas). However, based on field observations, this species is most commonly found in coral reef areas together with *P. longipes*.

Similar studies in Ceylon (de Bruin 1969), the South Pacific Islands (George 1972) and Thailand (Bhatia 1974) reported that *P. versicolor* and *P. ornatus* never enter traps even in areas where they are abundant unlike *P. penicillatus* and *P. longipes*. It is therefore interesting to note that these species are also occasionally caught in traps in eastern Samar.

George (1974) noted that *P. penicillatus* is the most likely species for commercial development in the Indo-West Pacific. Results in the present study indicate that this is true in eastern Samar. This species comprises over 80% of the total landed catch in the area. Thus, the lobster fishery in the area is mainly based on this species.

Although estimated CPUEs were higher in Station 7 than Station 6, there is no significant difference between all the monthly mean CPUEs for the two stations ($P < .05$). A decreasing trend in the monthly average CPUEs in both stations each year is notable. The highest CPUE estimates for both stations was in May of each year followed by a progressive decline towards the end of the "lobster season". Higher catches of fishermen during the start of the season may be attributed to minimized fishing pressure from October to April when the coastal waters are rough. Unfortunately, there are no records of the number of lobster fishermen in the past years to allow estimates of CPUEs from the total yearly landed catch (Table 4). CPUE estimates in this study for 1982-1984 in two stations suggest, however, a steady decline in the natural stock.

As mentioned earlier, there is a very competitive market for spiny lobsters. The fishery is an important source of income for many small fishermen throughout eastern Samar and revenue for the municipality of Guiuan. Thus, appropriate management measures must be considered immediately towards proper utilization of this valuable resource.

Spearfishing is unselective and does not provide an opportunity to regulate the catch. Thus, the regulation of spearfishing and at the same time the development of the existing trap fishery should be considered. In relation to the latter, better trap designs to increase efficiency should be looked into. A hand fishery for *P. penicillatus* exists in San Vicente, Cagayan (Gomez and Juinio; unpublished data) and it might also be worthwhile to assess the feasibility of adapting such practice in the area. Both the trap and the hand fishery will allow implementation of suitable management measures (prohibition on taking of egg-bearing females, minimum size limit) for the lobster resources in eastern Samar, Philippines.

Acknowledgements

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Table 1. Monthly frequencies of lobsters, and pooled relative percentages per species in sampled landed catch in Guiuan, E. Samar, November 1982-November 1983.

Month	Total no.	*P. penicillatus*	*P. longipes*	*P. versicolor*	*P. ornatus*	*P. homarus*
1982						
November	146	42	62	29	13	–
December	49	16	14	5	14	–
1983						
January	74	22	.22	13	17	–
February	477	178	130	142	27	–
March	215	93	31	76	15	–
April	2,845	2,444	229	61	111	
May		–	–	–		
June	488	471	13	4	–	–
July	5,120	4,581	451	81	4	3
August	2,099	1,769	243	77	8	2
September	1,133	919	141	58	15	–
October	207	119	45	37	6	–
November	54	13	16	19	6	–
Total	12,907	10,667	1,397	602	236	5
Relative %		82.3	10.8	4.7	1.8	–

Table 2. Monthly mean catch (kg*) of deliberate lobster spearfishermen per trip at Station 6, 1982-1984.

	1982			1983			1984		
	No. of fishermen	CPUE	s.d.	No. of fishermen	CPUE	s.d.	No. of fishermen	CPUE	s.d.
May	5	1.17	.42	9	1.04	.30	7	.90	.33
June	9	.88	.28	11	.72	.30	12	.57	.23
July	8	.73	.44	8	.58	.16	10	.56	.18
August	5	.56	.23	4	.46	0	8	.41	.01
Mean		.84			.70			.61	

*Total tail weight

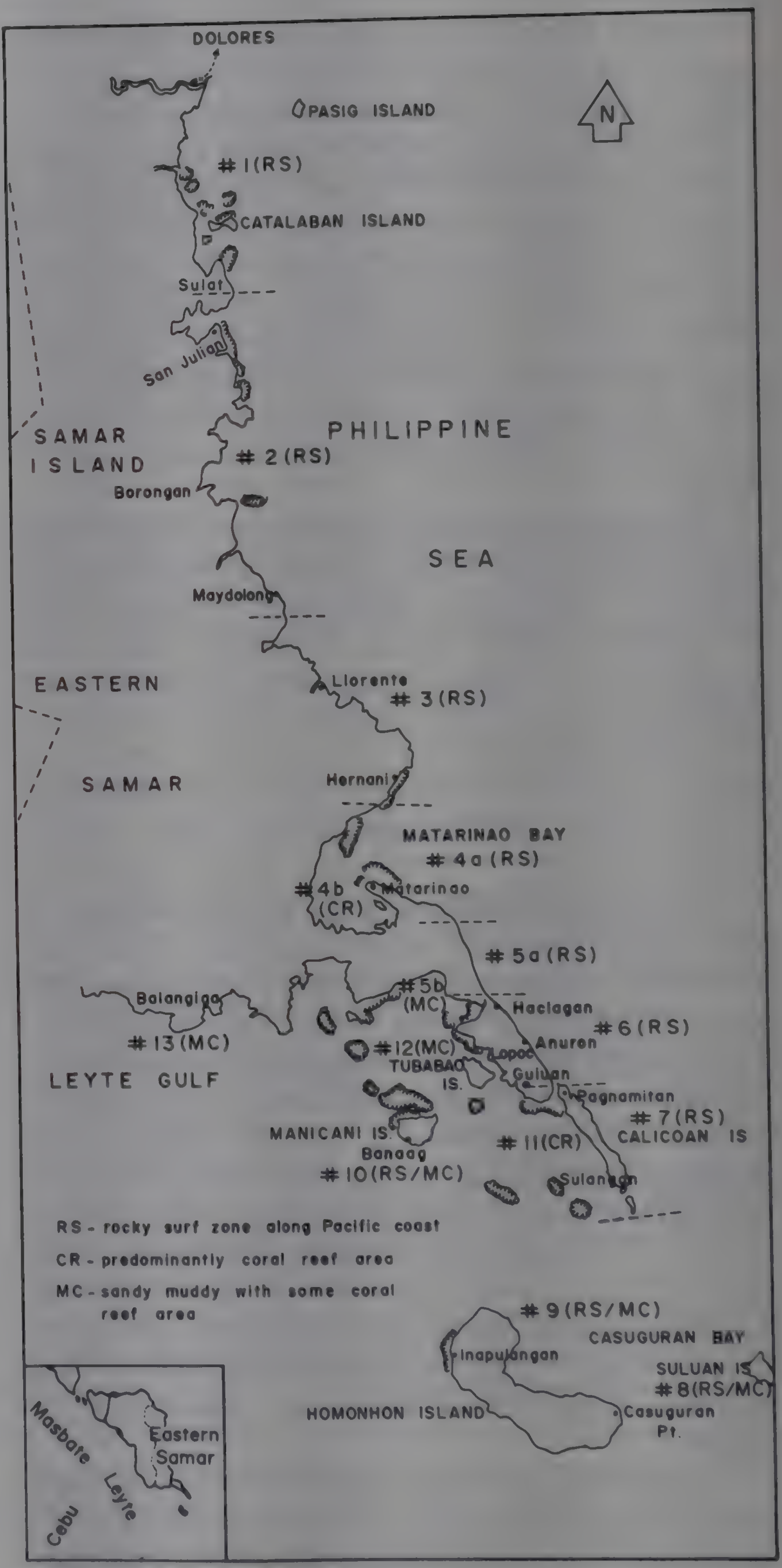
Table 3. Monthly mean catch (kg*) of deliberate lobster spearfishermen per trip at Station 7, 1983 and 1984.

	1983			1984		
	No. of fishermen	CPUE	s.d.	No. of fishermen	CPUE	s.d.
May	–	–	–	3	1.00	.25
June	3	1.12	.37	4	.74	.09
July	4	.78	.24	4	.67	.08
August	4	.74	.31	–	–	–
Mean		.88			.80	

Table 4. Yearly report of lobster shipout (kg) from Guiuan, Eastern Samar

Month	1973	1974	1975	1976	1977	1978	1979	1980	1981	1982	1983	1984
January	90	0	18	35	100	0	70	29	0	18	26	0
February	380	0	188	380	660	0	700	265	0	24	97	180
March	375	0	350	75	40	120	350	166	248	310	384	840
April	790	25	415	2,742	770	625	666	568	0	1,846	2,914	1,180
May	3,135	123	2,727	6,668	3,650	4,765	2,060	1,014	446	2,107	4,240	2,690
June	3,475	280	1,462	7,123	3,675	5,530	1,066	772	284	2,302	2,652	2,770
July	3,750	1,008	2,081	8,248	4,080	3,940	350	949	176	3,272	2,248	3,028
August	4,185	618	2,416	4,365	2,240	3,965	560	378	60	1,091	1,461	1,335
September	4,300	280	848	2,080	120	3,920	738	175	72	433	1,089	800
October	1,445	188	70	401	175	595	108	38	12	161	208	248
November	950	35	423	800	585	0	35	21	32	43	36	57
December	850	0	53	0	140	175	0	18	0	33	0	0
Total	23,805	2,434	10,821	32,907	16,345	23,635	6,718	4,390	1,318	11,440	15,323	12,828

Source: BFAR Auxiliary Invoice Records



DOLORES
PASIG ISLAND
N
#1(RS)
CATALABAN ISLAND
Sulat
San Julian
SAMAR ISLAND
PHILIPPINE
#2(RS)
Borongan
SEA
Maydolong
EASTERN
Llorente
#3(RS)
SAMAR
Hernani
MATARINAO BAY
#4a(RS)
#4b (CR)
Matarinao
#5a(RS)
Balangiga
#5b (MC)
Haclagan
#6(RS)
#13(MC)
#12(MC)
Anuron
LEYTE GULF
TUBABAO IS.
Lopoc
Guiuan
Pagnamitan
#7(RS)
CALICOAN IS
MANICANI IS
#11(CR)
Banaag
#10(RS/MC)
Sulangan
RS - rocky surf zone along Pacific coast
CR - predominantly coral reef area
MC - sandy muddy with some coral reef area
#9(RS/MC)
CASUGURAN BAY
Inapulangan
SULUAN IS
#8(RS/MC)
HOMONHON ISLAND
Casuguran Pt.
Masbate
Eastern Samar
Leyte
Cebu

Fig. 1. Thirteen lobster study stations and general types of lobster fishing grounds, Samar, Philippines.

An Overview of the Fisheries of San Miguel Bay, Philippines[a]

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Abstract

The paper presents a brief account of a multidisciplinary study from 1979 to 1982 in the 840-km^2 San Miguel Bay, Camarines Sur, Philippines, which covered the biological, economic and sociological aspects of the fishing industry.

A total annual fish production of about 20,000 t was recorded from the Bay. About 64% of this was contributed by some 5,100 small-scale fishermen, while the rest was landed by 95 commercial trawlers of varying sizes.

Economic analysis revealed the existence of strong competition among the different fishing sectors over the use of the Bay's resources and income is unevenly distributed in favor of the commercial trawlers, which employ only 7% of the total number of fishermen in the area. Ownership and earning of the trawlers were concentrated in 35 families, while the small-scale fishing gears were distributed evenly among 2,000 families. The sociological study revealed that there are very limited alternative employment opportunities around the Bay which results in the low income of the households and significant out-migration from the area.

Several alternative management measures were analyzed to help resolve the growing conflicts between the small-scale and commercial fishermen, whose landings were declining while fishing effort was increasing. The project proposed the establishment of the "San Miguel Bay Fisheries Authority" which should be responsible for defining management objectives for the Bay, collecting background information necessary for selecting appropriate management measures, as well as for ensuring their implementation.

Introduction

The present paper is based on the research project "Small-scale Fisheries of San Miguel Bay: A Multidisciplinary Analysis", which was conducted jointly by the Institute of Fisheries Development and Research (IFDR) of the University of the Philippines in the Visayas-College of Fisheries and the International Center for Living Aquatic Resources Management (ICLARM) from November 1979 to 1981.

The primary objective of the project was to conduct a multidisciplinary study in the 840-km^2 San Miguel Bay with emphasis on the problems of the "municipal" fishery and the fishing communities around the Bay (small-scale fishermen are in the Philippines, licensed by their municipalities, hence the name). It was recognized that biological, technological, economic and sociological factors all influence the income of municipal fishermen, and in order to fully understand their problems, the interrelationships of these factors must be determined. The project was the first attempt to use multidisciplinary approach in fisheries research in the Philippines. It was envisioned that such an approach could subsequently be used in other areas and that the results of the present study could serve as basis for government policymakers and planners to integrate the development of the fisheries of San Miguel Bay in its development program for the Bicol Region.

The Bay is characterized by a sandy mud substrate, with average depths of 5.25 m. The whole area is trawlable. Fishing is carried out all year, using different gears seasonally.

The overall project was divided into three distinct, but complementary modules: a) biology (stock assessment), which assessed physical constraints to fishing and the status of the fishery resources of the area; b) economics, which examined the catch, effort and incomes of the municipal and commercial fishermen as well as costs and returns for the major municipal fishing gears and economic efficiency of the marketing and distribution systems in the area; and c) sociology, which determined the nature and extent of the flow of human resources between municipal fisheries and other rural sectors; assessed the potential of programs that seek to reduce the dependence of households on capture fishing; examined the factors that influenced sharing systems for major gear types; and described the sociological aspects of the marketing systems.

Materials and Methods

Fourteen research personnel were fielded by the project, consisting of six members for the biological team, four for the economic team, and four for the sociology team. Three senior researchers guided the field personnel.

Some biological and hydrographic data were collected as background information on the assessment of the fisheries of the area. Available secondary hydrographic data were also used to describe the Bay's ecological character. A portable echosounder mounted on a motorized banca was used for a one-day bathymetric survey of the Bay.

The catch-per-effort by gear type was obtained by direct monitoring of fishing activities at selected landing places over a 12-month period. A team of research assistants also boarded trawlers twice each month to gather catch data during actual fishing operations.

An inventory of the fishing gears being used in the area was conducted by actual physical count of the larger gears, e.g., fixed gears, "baby" trawlers, etc., and through household surveys, using questionnaires and personal interviews of respondent fishermen in the case of smaller gears, like push nets and handlines.

Surveys of all possible sources of primary and secondary data, including published and unpublished reports, theses and raw data reports from various institutions were conducted to gather historical data on the Bay and its resources.

The data collection activities of the economic study team were divided into four phases: household inventories, landing and market surveys, costs and return record-keeping, and middlemen/processors survey. Either census or random sampling technique was used, depending on the size of the respondents.

Data for the sociological study were gathered over an 18-month period from 22 communities (total population = 40,000 or 3,690 households) bordering the San Miguel Bay area, involving a sample survey of 641 households and in-depth interviews of respondent active fishermen, fishing consumers, middlemen and processors. Data analysis was conducted mainly through cross tabulations of variables.

Results and Discussion

Pauly (1982) grouped the fish fauna of San Miguel Bay according to their types of habitats into:

a) Soft-bottom demersals inhabit the shallow, soft muddy portion of the Bay. This group of species predominates in the catch (55%). Examples are the Leiognathidae, Sciaenidae and Mullidae.

b) Hard-bottom demersals are associated with reefy/rocky substrates. About 20% of the ichthyofauna identified are probably inhabitants of the reef areas and rocky outcrops near the entrance of the Bay. Examples are the Serranidae, Lutjanidae and Chaetodontidae.

c) Coastal pelagics use the Bay as nursery area and comprise about 22% of the fish species found there. Examples are the Clupeidae and Engraulidae.

d) Oceanic pelagics enter the Bay occasionally for food or shelter but their young do not use it as a nursery area. Only 3% of the fish species caught belong to this group. Example are the large Scombridae.

The fish resources of San Miguel Bay are being fished by both commercial and municipal fishermen. The commercial fishermen mostly use trawlers of varying sizes. Vakily (1982) found there were 30 large trawlers ranging from 27 to 117 gross tons (GT) (which operated only occasionally inside the Bay) and are excluded from further consideration here; 20 medium trawlers, ranging from 3 to 6 GT; and 75 small trawlers ("municipal baby trawls") ranging from 2.5 to 2.9 GT.

The municipal fishermen were using a variety of non-trawl fishing gears (Table 1) and nearly 200 minitrawls. During the one-year period covered by the investigation, 19,133 t of fish, molluscs and crustaceans were landed from the Bay. Among the catch of the municipal fisheries are sergestid shrimps locally called "balao". These small crustaceans, represented in Philippine waters by *Acetes* sp., possibly *A. erythraeus* (Omori 1975), are caught by a special gear, the minitrawl, operated mainly from December to May. The "balao" fishery contributes about 23% (4,470) of the San Miguel Bay fishery.

About 64% (12,237 t) of the catch, including sergestid shrimps, were contributed by about 5,100 small-scale fishermen, while the remaining 36% (6,896 t) were landed by 95 trawlers, owned by 35 operators. The average catch of small-scale fishermen was thus 2.37 t while each commercial trawl operator took some 197 t yearly. The trawler catch had a total market value of ₱22 million while that of the small-scale fishing gears amounted to about ₱31 million (Smith et al. 1982).

The distribution of pure profits, defined as "resource rents above all costs" (Smith et al. 1982) from the catch (including "balao") by the different competing gear types in the Bay indicated that half the total pure profit of ₱3 million was earned by the trawlers alone, and the other half was distributed among the scale-fishing gears with the minitrawls getting the biggest share of ₱1 million, and the rest were shared proportionately by the gill netters (₱156,000), fish corrals (₱216,100) and filter nets (₱54,000). The Philippine government also earned a share of the resource rents amounting to ₱5.5 million in the form of its taxes on fuel. This tax was higher on nontrawl gears and minitrawls using regular gasoline than on trawlers which used diesel.

The study shows that the ownership and earnings of the trawlers were concentrated in only a few operators/families, while those of the small-scale fishing gears, including the mini-trawls were distributed rather evenly among a large number of families. Thus, the distribution of benefits from the Bay's fish resources is skewed in favor of the trawlers. The sociological study (Bailey 1982) revealed that there are very limited employment opportunities around the Bay, which results in low earnings of the households, prompting out-migration from the area. An annual increase of 2% in the number of fishermen around the Bay, was estimated, nevertheless.

Due to a rise in fuel costs, some commercial fishing boat operators shifted their operations to nearshore areas using smaller boats. It became apparent that the small-scale fishermen are faced with competition both from amongst themselves, as their number increases, as well as from trawlers.

Status of the San Miguel Bay Fisheries

The earliest survey conducted on the fishery resources of San Miguel Bay was done in 1935 (Umali 1937). Warfel and Manacop (1950) in their demersal otter trawl survey in 1947, found the highest density of fish among Philippine trawling grounds in San Miguel Bay. On this basis, they suggested that four to five trawlers could be maintained by the resources without any adverse effect. Following their survey, several fishing vessels started to operate in the Bay. Eventually, their number increased up to present levels, where the combined power of all crafts operating in the bay is 18,000 hp of which 13,200 hp are small and medium trawlers and 5,600 hp are small-scale crafts, including minitrawls and gill netters. The trawlable biomass in the Bay has declined to only 1,800 t, equivalent to a density of 2.13 t/km^2, only about 20% of the biomass that can be estimated from the data in Warfel and Manacop (1947) and Pauly (1982).

Pauly (1982) concluded that there is "ecosystem overfishing'" in the Bay, a condition in which the decline through fishing of the originally abundant stock is not fully compensated for by the increase of the biomass of other exploitable species. Thus the sharks, rays and slipmouths, which were once the major components of the trawl catch in San Miguel Bay, have now been largely replaced by croakers, squids and shrimps (see Belnas 1980 for catch effort data on the latter) which have smaller biomass than the group they replaced.

The following threats to the viability of the fishing industry of San Miguel Bay were identified:

1. There is biological overexploitation of the resources of the Bay, in the sense that an increase in fishing effort would not produce a corresponding increase in total catch.

2. Economic overfishing occurs in the Bay as evidenced by the fact that the rent represents only 6% of the gross value of the catch.

3. There is growing competition between the different fishing sectors in the Bay, which are exploiting the same resource. The trawlers, representing only 3% of the Bay's fishing units and employing only 7% of the total fisheries labor force in the area, are getting the largest share of the catch and 50% of the profit.

4. The entry into the fishery of both trawlers and the small-scale fishermen remains unregulated, resulting in declining catch per fisherman.

5. There are very few alternative employment opportunities for the fishermen around the San Miguel Bay area.

Management Options

The study revealed that there is a pressing need for management schemes for the fisheries of the Bay. The growing problem of overfishing and uneven distribution of incomes can only be minimized by limiting the amount of fishing effort. Increasing the 2-cm mesh size used by the trawlers and banning of commercial trawlers from municipal waters are considered useful measures, but enforcement of these restrictions appears difficult. Continued credit programs are unlikely to solve the problems of the small-scale fishermen unless steps are taken to regulate those gear types with which they compete.

It is critically important in this fishery that a management partnership be forged between the fishermen, local officials and concerned national government officials. The research team proposed the creation of a "San Miguel Bay Fishery Authority" (SMBFA) which would be responsible for setting management objectives for the whole Bay, collecting background information necessary for selecting management steps as well as implementing, monitoring and enforcing them. All fishermen would be encouraged to participate in decisionmaking by the SMBFA.

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[a] ICLARM Contribution No. 319.

Table 1. Small-scale gears used in the San Miguel Bay, with their Tagalog and Bikol names.

Gear type	Tagalog name	Name in San Miguel Bay area
Non-textile devices:		
Spear gun	salapang, panibat	antipara
Fish trap	bubo	bubo
Fish weir	pangharang	sabay
Stationary tidal weir		ambak
Fish corral	baklad	baklad, sagkad
Textile devices:		
Lines		
Pole and line	kawil	banwit
Longline	kitang	kitang
Nets		
Liftnets	panadiyok	bukatot
Scissor net	sakag	sakag
Crab liftnet	bintol	bintol
Filter net	dayakus	biakus
Beach seine	pukot	sinsoro
Minitrawl	–	itik-itik
Drift gill net	panti, paenod	panke
Drift gill net		palataw
Drift gill net		pamating
Crab gill net	–	pangasag
Bottom-set gill net	palagiang-paningahan	palubog

Fisheries and Fishery Management of Large Reservoirs in Thailand

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Abstract

The biological and physical features, and the fisheries management of major reservoirs in Thailand are described. At present 18 major reservoirs with a total surface area of approximately 230,000 ha are used for their fishery resources. The average annual yield was computed to be 73 kg/ha/year. Carps constitute a major group of the catches of all reservoirs followed by catfish and murrels. Fish stocking is the only effective management measure practiced in the reservoirs. *Oreochromis niloticus, Labeo rohita, Pangasius sutchi* and various types of Chinese carps are among stocked species which show a high degree of stocking success. Closed season, closed area, as well as limitation of damaging fishing methods are used in some reservoirs to protect fish stocks and to ensure adequate recruitment each year. Also, fishing techniques that cause damage to fish populations such as poisoning, electrofishing and use of explosives are seriously restricted. These various management techniques are discussed.

Introduction

Large reservoirs have become a major fishery resource of Thailand in the last two decades. Before, freshwater fish came mainly from flood areas of main river systems such as Chao Phaya and Maeklong. From 1960 to 1965, fish production from flood areas was estimated to be about 90% of total freshwater fish of the country (Larimore 1965). Now flood fisheries no longer exist because of the construction of dams across rivers and their tributaries. At present there are 18 major reservoirs which cover a total surface area of about 230,000 ha. The freshwater fishery resource then changed from flood areas to newly built reservoirs. As they are an important source of freshwater fish, the biological features as well as the fisheries of the reservoirs were studied to obtain necessary information for the formulation suitable management techniques. Management techniques have been practiced in some reservoirs as early as their completion. However, optimum yields from reservoirs have not yet been achieved. The aim of this paper is to describe the fisheries and fishery management concerned with fish production in Thai reservoirs.

Study Areas and Methods

Reservoirs for hydroelectric power generation and/or major irrigation systems are classified as large reservoirs in this paper. The reservoirs are located throughout Thailand (Fig. 1). Their surface areas range from 1,200 ha in Chulaporn to 41,000 ha in Ubolratana. Most are shallow and productive. There are ten reservoirs with mean depth not exceeding 10 m and five reservoirs of 11 and 16 m. Deep reservoirs are those of the hydroelectric type. They are located in mountainous areas such as Bhumipol, Sirikit and Srinakarin. Mean depth of these reservoirs exceeds 37 m (Table 1).

Great fluctuation of water level is common in Thai reservoirs. The loss of water is mainly through electric power generation, irrigation and evaporation. In shallow reservoirs about half of the surface areas are dried and the bottom soil is exposed to the sun for at least three months each year.

Water temperature of Thai reservoirs varies with climate. Normally, the temperature of northern reservoirs is lower than those in other parts of the country. It ranges from 20°C during winter in the north to 32°C during summer in the northeast.

Water turbidity is a remarkable physical feature of Thai reservoirs. Soil erosion is the major cause of water turbidity. Turbidity was found up to 258 units upstream of Ubolratana reservoir as against less than 1 unit during the normal period. High turbidity occurs after rainfall and remains for two months after the rainy season. All reservoirs, especially those in the northeast where forests in the watershed have been largerly destroyed, have turbidity problems.

Results

Thai reservoirs are productive, comparable to those of productive lakes in other tropical regions. The primary production of five reservoirs, ranging from 1,500 to 2,600 mgC/m^2/d (Srisuwantach and Soungchomphan 1981) is comparable to 1,700-3,800 mgC/m^2/d in Lake Victoria of East Africa, 400-5,000 mgC/m^2/d in Lake Lanao, Philippines and 1,350-4,050 mgC/m2/d in reservoirs of East Java, Indonesia (Talling 1965; Lewis 1974; Henard 1979, respectively). Morphoedaphic indices (MEI) derived from conductivity and mean depth were from 4.1 in Sirikit to 24.1 and in Ubolratana (Table 2). The index was correlated with fish yields and the following equation was obtained.

$$Y = 1.28 + 0.3696\,X \quad (r = 0.423)$$
$$Y = \log \text{fish yield},\ X = \log \text{MEI}_c$$

where MEI_c = conductivity/mean depth.

The yield of nine reservoirs was correlated with MEI derived from total alkalinity. The following yield equation was obtained with improved correlation coefficient:

$$Y = 1.511 + 0.3698\,X \ (r = 0.5246)$$
$$Y = \log \text{yield (kg/ha/year)} \quad X = \log \text{MEI}_a$$

where MEI_a = alkalinity/mean depth.

The number of species of fish in Thai reservoirs varies from 17 in Bangpra to 94 in Ubolratana (Table 2). Species of fish in the reservoirs depend on the original fish within the impounded area before being flooded. After the closing of the dam, the number of fish species may decrease as in many reservoirs; for example, eight species of barb and catfish were not present in the samples of fish fauna studied after four years of closing Ubolratana reservoir. Some species, such as *Anabus*, *Botia*, *Trichogaster* and *Clarias* can survive in reservoir environments but with less degree of spawning success. There are minor groups of fish in all reservoirs.

Of the total native species of each reservoir, less than 50% are frequently encountered in the catch and are economically valuable. There are four species commonly found in Thai reservoirs: *Channa striata*, *Osteochilus hasselti*, *Notopterus notopterus* and *Mystus nemurus*. The following species are found in all reservoirs of all regions except those in the south: *Osteochilus melanopleura*, *Corica goniognatus*, *Puntius gonionotus*, *Morulius crysophegadion*, *Kryptopterus* spp., *Oxyeleotris marmoratus* and *Cirrhinus jullieni*. Carp were found to be the most numerous, followed by silurids and cobitids with respect to number of species in all reservoirs.

Annual yields of Thai reservoirs are shown in Table 2. Great variation of fish production, either among the reservoirs or within each reservoir from year to year is caused by many factors such as productivity of the reservoir, number of fishermen, fishing techniques and management policy. Shallow reservoirs with wide fluctuations provide high fish production. Normally, high fish yield is obtained one year after high water level (Bhukaswan and Pholprasith 1976).

One remarkable phenomenon in Thai reservoirs is the explosive growth of the clupeid, *Corica goniognatus*. A considerable amount of this species was first observed in Ubolratana reservoir in 1974 when it contributed 450 t or 25% of the total catch of the reservoir. *Cirrhinus jullieni* is another pelagic species that plays a great role in fish production of the reservoirs. In Ubolratana reservoir this fish has comprised about 24 to 36% of total catch since 1976. Recently both species appeared in other large reservoirs. They contributed 18.4, 52.4 and 62.3% of total catches of Sirikit, Sirinthorn and Lampao, respectively.

Fishing techniques are similar in all Thai reservoirs. Partial removal of timber and underwater stumps necessitates fishing gears of the stationary type. Gill net is the most popular and effective. Other fishing gears include the long line hook, scoop net, dip net, cast net and giant lift net. The lift net is often operated in conjunction with a gill net to increase its harvest efficiency. Scoop nets with light attraction are used for *Corica*, a plankton feeder. Fishing for *Corica* is done in the morning when fish are schooling. Paddle boats and long-tailed motor boats are favorite transportation in all reservoirs.

Several fishery management techniques have been introduced into Thai reservoirs to obtain optimum fish yield and to conserve fish. The following are most effective and suitable techniques for Thai reservoirs.

Fish stocking. Millions of fingerlings of different desirable species have been introduced into Thai reservoirs since 1965. The main purpose of stocking is to increase fish production. Introduced fishes are those of fast growing species and those that occupy ecological niches not used by local species. *Oreochromis niloticus*, *Labeo rohita*, *Pangasius sutchi*, *Puntius gonionotus* and Chinese carp are favorite introduced species. The grass carp reached 12.4 kg in two years, silver carp 5.8 kg in one year and rohu 5.4 kg in three years. Only *O. niloticus* and *P. gonionotus* have spawned naturally in the reservoirs of all regions.

Up to 1985, over twenty species of fish have been introduced into Thai reservoirs. *Ctenopharyngodon idella*, *Tilapia rendalli* and *Osphronemus goramy* were introduced for weed control especially during the early stage of the reservoirs. However, the degree of weed destruction by fishes has not yet been determined. *Probarbus jullieni*, *Pangasianodon gigas*, *Pangasius snitwongsei* and *Danoides microlepis* were stocked for conservation purposes. The first three species are common in the Maekong river system. After the successful artificial breeding of natural stock, their fingerlings were introduced into water resources throughout the country. The fish showed a good growth rate but reproduction has not been reported.

Macrobrachium rosenbergii is the only invertebrate with good growth and recovery rate in large reservoirs. About 300,000 fry were stocked into Ubolratana reservoir in 1982. Over 2 t of market-size prawns (300-500 g) were recaptured one year thereafter (Pawaputanon 1983). The experiment was repeated by Benchakarn in 1985 and a similar result was reported. At present, giant prawn, *O. niloticus* and *L. rohita* have become the daily landing species of Ubolratana reservoir.

Control of fishing. Fishing in Thai reservoirs is carefully regulated. Gill nets, cast nets, long line hooks and lift nets need not be regulated because they cause no damage to fisheries. However, fishing gears that are very effective and may cause damage to fish populations such as trawling with a motor boat, purse seine, surrounding net and blocking net are prohibited. Electrofishing, poisoning and using explosives are very seriously restricted in all reservoirs.

Closed season and closed area. Fishing is allowed with some restriction during the spawning season (16 May-15 September). The duration of restriction varies with location and certain circumstances. Reservoirs where spawning areas are located are closed to fishing during the spawning period and the two months thereafter. This is to protect young fish in the nursery area until they grow large enough to leave the nursery.

Discussion and Recommendations

Large reservoirs are now a major freshwater fishery resource of Thailand. Fisheries in the reservoirs have not yet been fully developed although some fish of Thai reservoirs are large herbivores and carnivores of economic importance. Low fish yields of Chulaporn, Namoon and Krasiew are due to low fishing density; the reservoirs are located too far from villages and transportation is not convenient. Thus, fisheries in these reservoirs are not fully utilized.

The status of the fisheries in Thai reservoirs cannot be analyzed collectively because necessary information is not available. Yield equations may not be valid because of inadequate data of fish landings. Widely dispersed fishing activities are the cause of the unreliability of data on the estimated yield of the reservoirs. The only reliable statistics are obtained from Ubolratana reservoir where the catch has been recorded daily since the opening of the reservoir. Similar recording was also made in Lam Pao and Kangkrachan but only in the first few years. The yield of other reservoirs (Table 2) was estimated from either creel census or area density methods which may differ from actual catches. However, the equations may be used to predict the production of any of the reservoirs of similar conditions. Fishing efforts should be recorded and standardized to obtain catch per unit of effort to determine yield.

The management of Thai reservoirs seem effective and economical at present. However, some techniques like water level manipulation can be improved. Normally water level cannot be manipulated only for fishery purposes which are secondary to other uses. Altering water level may affect water use downstream and flood control capacity. But maintenance of water at certain levels for some period of time to enhance survival and growth of commercial species is possible. This may require precise knowledge of the spawning period.

The presence of too many species of fishes in the reservoir is another factor that makes fishery management difficult. Effective management of a particular species can affect other valuable species. Too many species may reach their maturity at different sizes, which makes size selection impossible. Closing reservoirs to fishing during the spawning season may not work if the parent stock of valuable species is overfished during the fishing season, especially when water in the reservoir is at a minimum level. At such times fishes are easy to catch and need more protection, such as a fish sanctuary during the dry season.

Fish stocking programs of Thai reservoirs are not yet well planned. The number and species of fish and the time to stock particular reservoirs are not clearly programmed in advance. Stocking is made whenever fingerlings are available. Thus, thousands of the same species of different size may be stocked in one reservoir several times during the year. The only evaluation for this type of stocking practice is to estimate the amount of fish recovered over a period of time. Growth and survival rates, however, cannot be computed from the recovered fish unless tagging or marking is carried out.

The recovery rate of introduced fish has been less than 1%, which may be due to the small size of fish stocked. In reservoirs where several carnivore species exist, the fish to be stocked should be larger than 13 cm. However, predator-prey relationship within the reservoir should also be clearly understood so that size, species and number of fish to stock can be effectively estimated.

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Table 1. Morphometric and hydrographic features of major reservoirs in Thailand.

Reservoirs	Province	Year built	Surface area (ha)	Maximum storage $m^3 \times 10^6$	Mean depth (m)	Purposes
North						
Bhumipol	Tak	1965	30,000	13,400	45	HI
Sirikit	Utaradith	1872	28,500	10,540	37	HI
Kew Lom	Lampang	1972	1,600	80	5	I
Northeast						
Ubolratana	Khonkan	1965	41,000	2,550	6.2	HI
Sirinthorn	Ubolrachatani	1971	29,200	1,500	5.5	HI
Chulaporn	Chaiyapum	1972	1,200	192	16	HI
Nam pung	Sakon Nakorn	1966	2,000	165	8.6	HI
Nam Oon	Sakon Nakorn	1973	8,600	520	6.4	I
Lam Takong	Nakorn Rachasima	1968	4,400	445	16	I
Lam Praplerng	Nakorn Rachasima	1969	1,860	148	11	I
Lam Pao	Kalasin	1968	23,000	1,160	5.6	I
Central						
Kang Krachan	Pachburi	1966	4,960	710	14.3	HI
Srinakarin	Kanchanaburi	1978	40,000	16,800	42	HI
Pranburi	Prachuap Kiriken	1976	4,670	467	10	I
Bangpra	Cholburi	1975 (improve)	1,760	123	7	I
Krasiew	Supanburi	1980	4,800	240	5	I
Doklai	Rayong	1975	1,280	64	5	I
South						
Banglang	Yala	1980	4,500	720	16	HI

H = Hydroelectric
I = Irrigation

Table 2. Morphoedaphic index (MEI_c), gross photosynthesis (PG) and fish yield (FY) of major reservoirs in Thailand.

Reservoir	MEI	PG gC/m/d	FY (t/year) Min.	FY (t/year) Max.	Average kg/ha/year	No. of species
Bhumipol	3.33	–	548	1,108	37.0	69
Sirikit	4.10	1.5	506	1,343	47.2	76
Kew Lom	–	–	50	200	78.1	22
Ubolratana	24.10	2.4	1,213	2,598	61.0	94
Sirinthorn	–	–	1,202	3,313	113.5	56
Chulaporn	–	–	–	46	37.5	20
Nam Pung	–	–	–	131	66.2	21
Nam Ooon	–	–	103	197	23.0	70
Lam Takong	–	–	99	227	62.5	31
Lam Praplerng	–	–	141	252	135.5	32
Lam Pao	–	–	497	1,670	72.6	69
Kang Krachan	8.00	2.1	144	362	73.0	61
Srinakarin*	5.50	2.1	218	570	14.5	26
Pranburi	9.70	2.6	–	330	70.6	–
Bangpra	–	–	–	426	241.6	17
Krasiew	–	–	–	162	31.6	34
Doklai	–	–	–	99	77.8	–
Banglang	–	–	–	–	–	42
			Total	13,371	Average	73.24

*Reservoir was still filling, only at 20% of total capacity

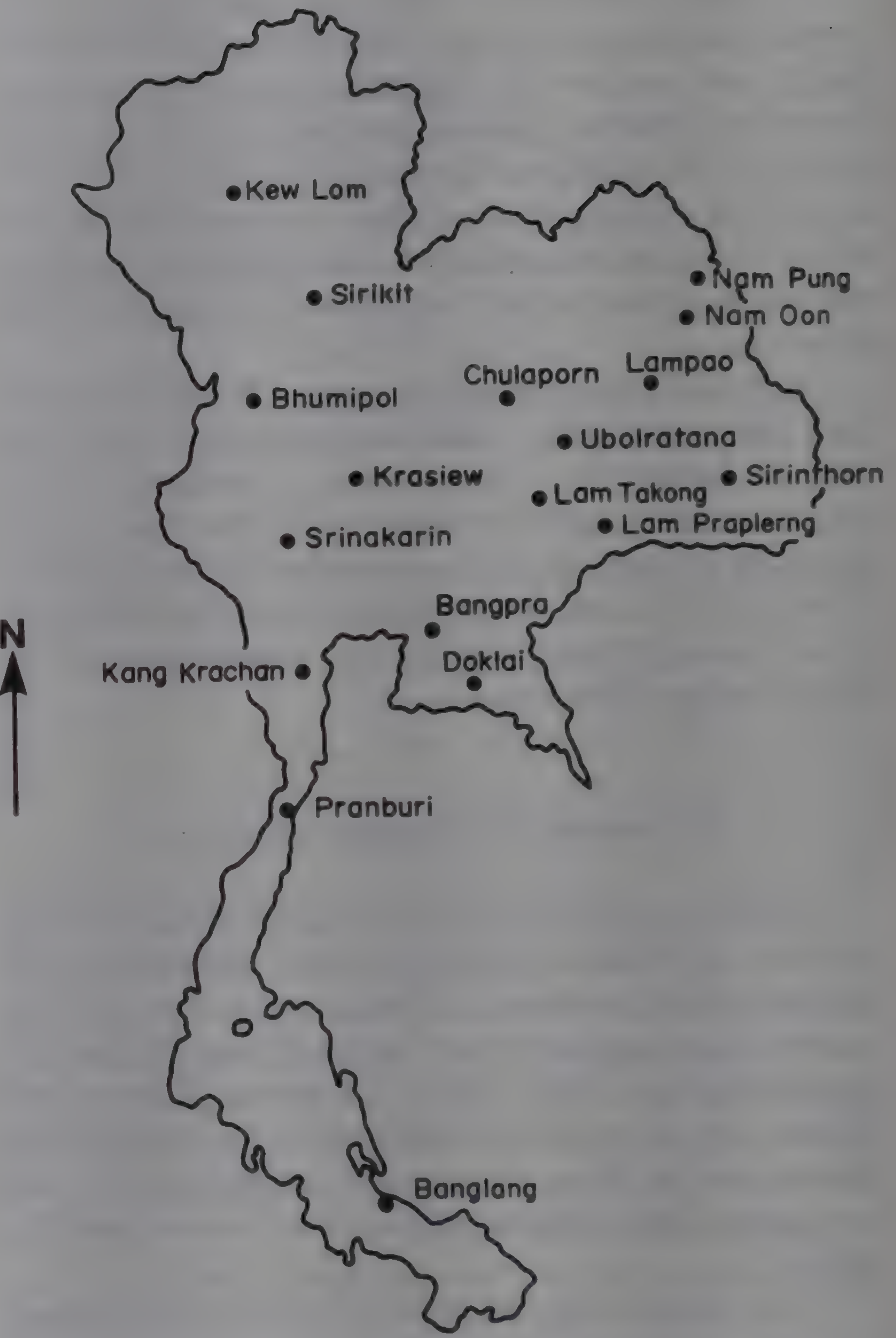

Fig. 1. Map of Thailand showing the location of major reservoirs.

The Fishery of the Giant Freshwater Prawn *Macrobrachium rosenbergii* (de Man) in Sri Lanka

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Abstract

Catch statistics for *M. rosenbergii* were collected from the three main *Macrobrachium* fishery areas in Sri Lanka - Panadura, Katunayake and Chilaw - for one year from January to December 1980. The peak catches were recorded during April to June and October to December. More females than males were present in the catches. Statistical analysis showed that the peak catches and the presence of a greater number of berried females in the catches were positively correlated with rainfall. In all the areas investigated the dominant sizes of prawns were 15 to 25 cm. The present production of *M. rosenbergii* from these areas was estimated to be about 6,000 kg/year.

Introduction

Although the bionomics and the fishery of *Macrobrachium rosenbergii* (De Man) have been studied by several workers in the South Asian region, such as in India (John 1957; George 1969) and in Bangladesh (Patra 1977), attempts have not been made to assess the possible magnitude of the fishery of this prawn in Sri Lanka which is still not well organized and is mainly at a subsistence level.

The fishing methods employed in the *Macrobrachium rosenbergii* fishery in Sri Lanka are more or less similar to those used in other countries of the Indo-Pacific region and are based upon indigenous gears (Ward and Wyman 1975).

These studies were undertaken to get an idea of the seasonal variation in abundance of this prawn, the sex ratios of the adult natural populations and the status of the present fishery in Sri Lanka.

Materials and Methods

Catch statistics for *M. rosenbergii* were collected throughout 1980 from the three main fishing areas in Sri Lanka: Panadura, Katunayake and Chilaw in the southwest part of the island. The total weight, total length, carapace length and sex ratio were determined from random basket samples collected at landing sites. The rainfall data in respect to each sampling station were collected from the Department of Meteorology, Colombo.

Results

Total catches of male and female *M. rosenbergii* landed at Panadura, Katunayake and Chilaw from January to December 1980 by weight are shown in Fig. 1.

The total catch shows two clear peaks around May and November. The minimum catches occurred during January to March and July to September. The number of females caught from all three areas was greater throughout the year than the males. The total catch, both males and females from Panadura, Katunayake and Chilaw was estimated to be 2,455, 2,485 and 1,049 kg, respectively, during the year of study. In all areas the highest catches coincided with periods of high rainfall (Fig. 1). Statistical analysis showed a positive correlation between total monthly catch and the amount of rainfall significant at 5% level ($r = 0.38$, $n = 36$) when the catches of all three sampling areas were considered together.

The seasonal variations of the distribution in numbers of male and female prawns in relation to rainfall with respect to Panadura, Katunayake and Chilaw areas are shown in Fig. 1. In general the number of female prawns caught was greater during the rainy months, the average ranging from 60 to 90%.

The numbers of berried females caught in relation to rainfall are given in Fig. 1. More berried females occurred during months of high rainfall. The statistical relationships are given in Table 1.

For convenience the prawns caught were considered under seven length groups. The frequency of occurrence of these is given in Fig. 2. In all the areas sampled the catch consisted mostly of prawns ranging in length from 15 to 25 cm. When the sexes are considered separately, however, the females in the catch generally ranged from 10 to 24 cm while males ranged from 20 to 34 cm.

Discussion

These catches were collected from the three main *M. rosenbergii* fishing areas in Sri Lanka on the southwest quarter of the island. There is no established fishery in the other areas of the island. The southwest part of the island receives rainfall during the southwest monsoon from May to September as well as intermonsoonal rains from October to December. The analysis of the catch shows that the peak catches coincide with months of high rainfall. During these times the salinity falls to almost zero levels in the lagoons (Costa and Fernando 1981; Silva 1981). The presence of a high percentage of females (about 60-90%) including berried females in the landings during the rainy months tends to support a migratory movement towards the lagoons during these months. Such migratory patterns have been already described for *M. rosenbergii* and other *Macrobrachium* species (John 1957; Raman 1967; Ling 1969; Hughes and Richards 1973; Costa 1979). Rainfall lowers the salinity downstream thus enabling the adult prawns to move closer to the mouths of the rivers resulting in greater numbers of prawns being caught.

The minimum catches were recorded during the months of February-March and July-August. This could be associated with lower rainfall. During low rainfall periods the salinity of the waters downstream is higher, over 17 ppt. The sex ratio was about 1 male to 3 females in the catches in all three areas. Smith et al. (1978) have indicated that the sex ratio of this species was generally biased towards females. Since the majority of the landings are in downstream areas closer to the mouths of rivers there is a greater chance of encountering females migrating seawards for spawning.

The annual contribution of *M. rosenbergii* to the fishing industry in Sri Lanka seems to be very small. Although there has been a greater demand for *M. rosenbergii* in recent years there is a dearth in the local markets. Many reasons could be attributed to this situation. Lack of a well organized fishery for *M. rosenbergii*, small populations existing in natural waters, high predation both during juvenile and adult stages and variations in rainfall may all serve as possible contributory factors for this underdeveloped *M. rosenbergii* fishing industry in Sri Lanka.

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Table 1. Statistical relationships between rainfall (X) and % of berried females in the catch (Y).

Area	Statistical relationship	Correlation coefficient
Panadura	$Y = .0402 X + 11.9319$	.8321*
Katunayake	$Y = .0471 X + 10.8417$	.9116*
Chilaw	$Y = .0652 X + 10.6190$	.7854*

*Values significant at 5% level; n = 12.

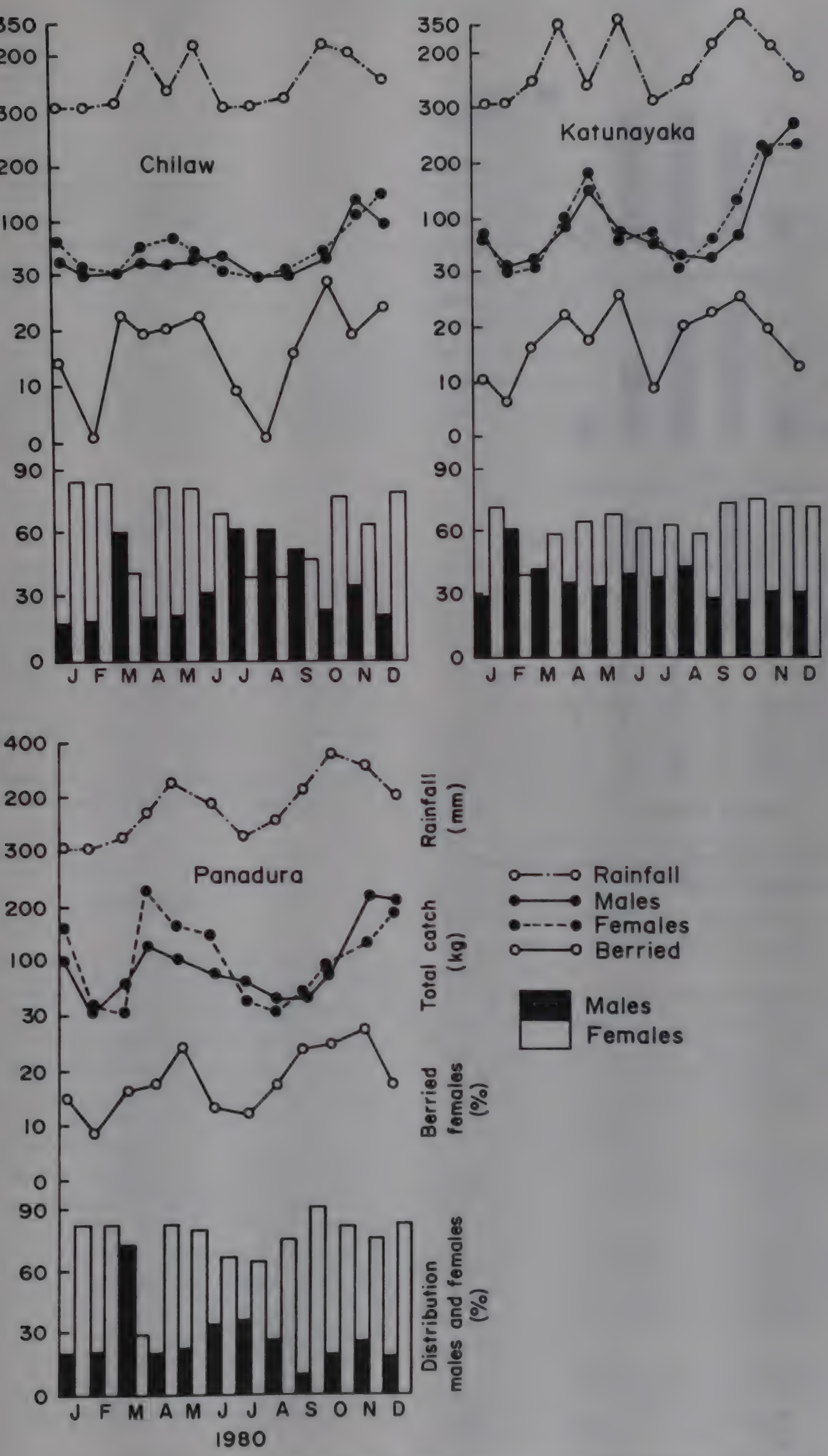

Fig. 1. Relationship between rainfall and total catch, % berried females and distribution of males and females in Chilaw, Katunayaka and Panadura lagoons.

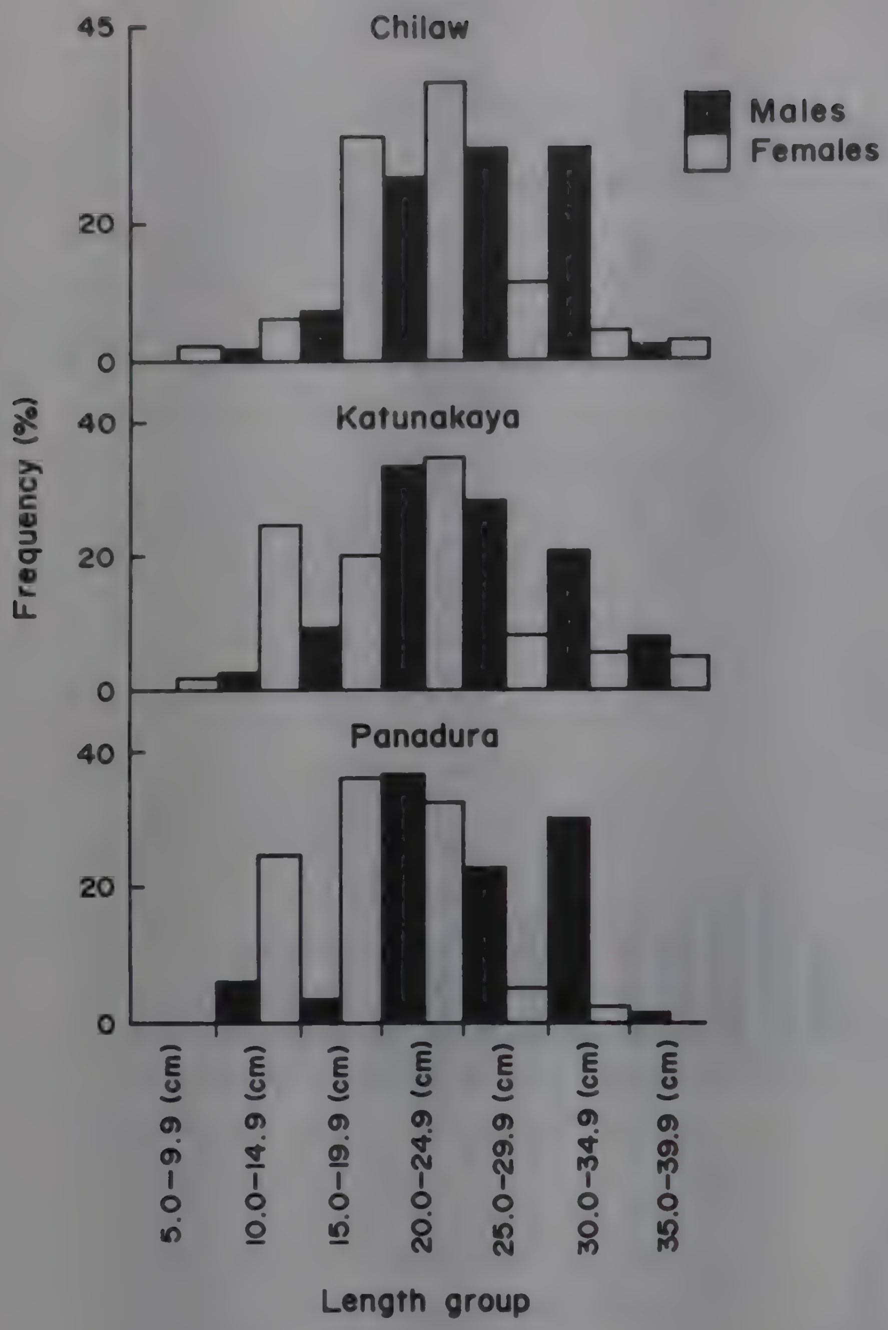

Fig. 2. Length-distribution frequency of *M. rosenbergii* (males and females) at different areas, 1980.

The Pot Fishery of Lake Biwa, Japan

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Abstract

Lake Biwa with an area of 676 km^2 is Japan's largest body of freshwater where various fishing gears have long been operated for its fisheries. One major fishery of the lake is the pot fishery which uses three types of pots designed for catching carp, crucian carp and prawns. In recent years, the catch of the pot fishery shows a decreasing trend and so with the total catch in the lake. Consequently, the number of licensed pot operators has decreased. With the pot target species also being harvested with the use of other fishing gears, the catch contributed by pot fishing has decreased relatively. The major lake fisheries are presented with emphasis on the pot fishery, using available statistical data to show how a traditional fishery has survived with increased fishing pressure on a freshwater lake.

Introduction

Lake Biwa, which is located in Shiga prefecture, is the largest lake (672 km^2 with 103.8 m maximum depth) in Japan. Presently, 24 species of fishes (including six endemic species), five species of shellfish and two species of prawns are fished commercially (Maehata et al. 1984). The fishing gears used in the lake are trawl nets, dredge, bamboo screen, gill nets, cast nets, dip nets, pot, beach seine, small long line and others.

The total catch in Lake Biwa has declined since 1973 and further decreased since 1976 from the level of 5,000 t with exception in 1981. The catch by pot fishery has also declined in 1974 and in 1983, it is almost 50% of the highest in 1972. The catch by pot fishery is the fifth among the major fisheries in 1983, i.e., 1) trawl nets and dredge (1,947.8 t); 2) bamboo screen labyrinth (994.1 t); 3) gill nets (807.2 t); 4) special type of dip net (428.4 t); 5) pot (397.8 t); 6) weir (269.8 t); 7) beach seine (184.5 t); 8) dip net (168.5 t). The pot fishery contributed about 8% to the total catch (5,046.7 t). If the number of licensed fishing units is considered, the contribution of pot fishery to the total (2,856 units) is about 23%. Licensed units have the permission of the prefectural governor or the Minister of Agriculture, Forestry and Fisheries based on a prefectural rule or ministerial rule which is further based on the Fisheries Law and Fisheries Resources Protection Act of Japan. In Lake Biwa, the pot fishery is one of the important fisheries. This report provides information on its status.

Fishing Gear and Methods

Various sizes and designs of pots are used depending on the target species. Basically, these pots are classified into three types (Board of Education of Shiga Prefecture): (1) Prawn pot, "Ebi-tatsube", (2) Basket trap I, "Funa-tatsube" and "Koi-tatsube", (3) Basket trap II, "Sen", "Ue" and "Moji".

Prawn pot. Cylindrical pots are used extensively to catch oriental river prawn *(Macrobrachium nippones)* and striped prawn *(Palaemon paucidens)*. A typical pot is usually made from split bamboo (Fig. 1). When pots are used to capture oriental river prawn, the opening of the funnel is larger than when used for striped prawn, but both depth and diameter of opening differ with each fisherman. Recently, the use of plastic material has increased, requiring less maintenance cost than bamboo. Generally, the construction of plastic pot is the same as the traditional bamboo pot.

In practice, 100 pots are fastened by ganging line (Fig. 1). The series of pots forms one unit or set. Many sets, often over 10, are employed in fishing. Baits vary according to the choice of the fisherman. Dumplings made of powdered chryslis mixed flour, salt fish flesh and fish viscera are generally used.

The fishing period is mainly from April to August for striped prawn and September to November for oriental river prawn.

Basket trap I. Traditional traps, used to capture carp *Cyprinus carpio* and crucian carps *Carassius auratus grandoculis* and *C. auratus cuvieri* are made from split bamboo, are cylindrical in shape and with one entrance at the side (Fig. 2).

When traps are used to catch carp, they are called "Koi-tatsube". The traps are sank with bait (Fig. 2). The fishermen mainly use vegetable bait such as dumpling made of rice bran mixed with flour, or boiled sweet potato and flour. Generally, the fishing season is July-November.

When the traps are used to capture crucian carps, they are called "Funa-tatsube". The construction and the size are almost the same as "Koi-tatsube". The traps are set without bait. Because the operation is done mainly during the spawning season of the species, the traps are usually set inshore with the upper portion protruding from the water surface (Fig. 2), the entrance facing the shoreline. Fishing period is from March to June which coincides with the spawning season of the crucian carps.

Basket trap II. Originally, these traps often called "Moji", "Ue" and "Sen", were woven out of bamboo with similar basic construction but the size and operation method vary according to the sizes and habits of the target species (Fig. 3). Large types are also used to capture carp and crucian carps and small types to catch oily gudegeon, *Sarocheilichthys biwaensis*, bagrid catfish, *Pelteobagrus nudiceps* and lake goby *Rhinogobius brunneus*. In recent times, these small traps have not been used widely.

Catch by Pot Fishery

The total catch from Lake Biwa has gradually decreased since 1973 (Table 1) to 5,046.7 t in 1983, which is about 60% of the total catch at peak level in 1972. The catch of pot fishery shows decreasing trend in recent years (Table 1), although the gears have improved in the materials used and to a small extent new types of gears are being developed.

For the last 10 years the catch by pot fishery has also declined; the catch in 1983 is less than 50% of the catch at the peak level (Table 1). In terms of catch by type of fishing gear, the drop in catch by prawn pot is considerably large. The catch contribution of prawn pot to the total catch of pot fishery is about 80%, thus the decreasing trend is due to the decrease in the catch by prawn pot.

In terms of the main target species (Table 2), from 1975 to 1982 the catch contribution of striped prawn was considerably larger than of the other species. Thus, the drop in the catch due to the decrease in the catch of striped prawn is significant.

As these species are also caught by other fishing gears, it is necessary to examine in detail the variation in the catch according to the type of fishing gear. Carp and crucian carps are caught mainly by gill nets (Fig. 4), so that the contribution of the catch by pots for these species is low. In recent years, the catch of these species has decreased and projection indicates this trend may continue.

Prawns are caught by three types of fishing gears, i.e., bamboo screen, trawls and pot (Fig. 4). Almost all catches of oriental river prawn are by the use of prawn pot. It is necessary to keep this prawn whole and good body shape for higher sale value because, head on, it is one of the highest priced among prawns. For the last five years the market price of this species ranked third among the fish prices (yearly mean) in the fish market: 1) sweet fish (fry); 5,742 yen/kg; 2) sweet fish, 3,791 yen/kg (all of the catch is distributed as seed for aquaculture); 3) oriental river prawn, 3,326 yen/kg in 1982.

On the other hand, the contribution of the striped prawn catch using pots is about 40% (Fig. 4). This species is also caught by other fishing gears, such as trawl and bamboo screen, contributing almost 60% to the total catch. It is very difficult to relate the catches by fishing gears because of the large variation in the trawl catches and the increasing catches by bamboo screen in recent years. The decrease of the catch by pot may be due to the catch by other fishing gears.

As already mentioned, the pot fishery at present is not in a good state. The number of licensed pots has declined since 1980 (998 units) with the decrease in catch by the pot fishery; in 1985, the licensed pots number 483 which is less than 50% of those licensed at the peak level in 1980.

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Table 1. Catch by type of fishing gear, in tonnes, Lake Biwa, 1970-1983. (Source: Annual Report on Fisheries, Shiga prefecture).

Year	Total catch	Catch by pot (t)	Prawn pot		Basket trap I		Small fyke net	
			(t)	(%)	(t)	(%)	(t)	(%)
1970	6,459.8	352.6	262.5	(74.5)[a]	68.8	(19.5)	21.3	(6.0)
1971	6,383.4	401.0	273.4	(68.2)	90.4	(22.5)	37.2	(9.3)
1972	8,075.0	654.1	533.9	(81.6)	86.7	(13.3)	33.5	(5.1)
1973	6,742.6	561.0	478.5	(85.3)	58.6	(10.4)	23.9	(4.3)
1974	6,415.7	828.8	705.8	(85.1)	75.1	(9.1)	47.9	(5.8)
1975	6,002.6	723.9	613.7	(84.8)	69.7	(9.6)	40.5	(5.6)
1976	5,428.8	698.1	620.0	(88.8)	50.9	(7.3)	27.2	(3.9)
1977	5,450.7	530.0	446.3	(84.2)	57.7	(10.9)	26.0	(4.9)
1978	5,491.6	471.8	392.3	(83.1)	53.0	(11.2)	26.5	(5.7)
1979	5,288.4	359.5	285.1	(79.3)	46.9	(13.0)	27.5	(7.7)
1980	5,189.2	325.4	256.3	(78.5)	46.2	(14.2)	23.9	(7.3)
1981	4,918.5	533.6	480.5	(90.0)	37.8	(7.1)	15.3	(2.9)
1982	5,059.4	435.9	383.5	(88.0)	35.4	(8.1)	17.0	(3.9)
1983	5,046.7	397.8	340.9	(85.5)	–	–	56.9	(14.5)[b]

[a] Numbers in parentheses represent the percentage to the catch by pot fishery.

[b] Including catch by basket trap I.

Table 2. Catch by pot for main target species (t), 1975-1982. (Source: Annual Report on Fisheries, Shiga prefecture).

Year	Carp	Crucian carps	Oriental river prawn	Striped prawn
1975	202.1 B:24.1 F:10.4	616.5 B:38.0 F:26.6	27.3 P:24.3	1,301.2 P:589.3
1976	170.5 B:20.4 F: 5.6	573.0 B:34.8 F:20.6	18.4 P:15.6	1,445.9 P:604.4
1977	149.4 B:16.7 F: 6.1	693.3 B:34.8 18.5	15.8 P:11.9	1,080.3 P:434.3
1978	142.7 B:12.3 F: 3.9	577.5 B:34.1 F:19.7	25.4 P:21.0	841.9 P:371.0
1979	148.7 B:14.2 F: 5.8	633.4 B:29.1 F:19.3	31.1 P:28.5	716.6 P:256.6
1980	164.0 B:14.8 F: 6.9	791.1 B:26.4 P:14.7	20.5 P:18.3	704.0 P:236.8
1981	163.0 B: 8.1 F: 2.6	875.3 B:25.7 F: 9.7	13.2 P:12.0	983.0 P:468.5
1982	138.1 B:10.6 F: 2.5	741.8 B:22.4 F:12.0	18.6 P:17.4	940.5 P:366.0

The upper column for each year shows total catch of each species; B, catch by basket trap; F, catch by fyke net; P, catch by prawn pot.

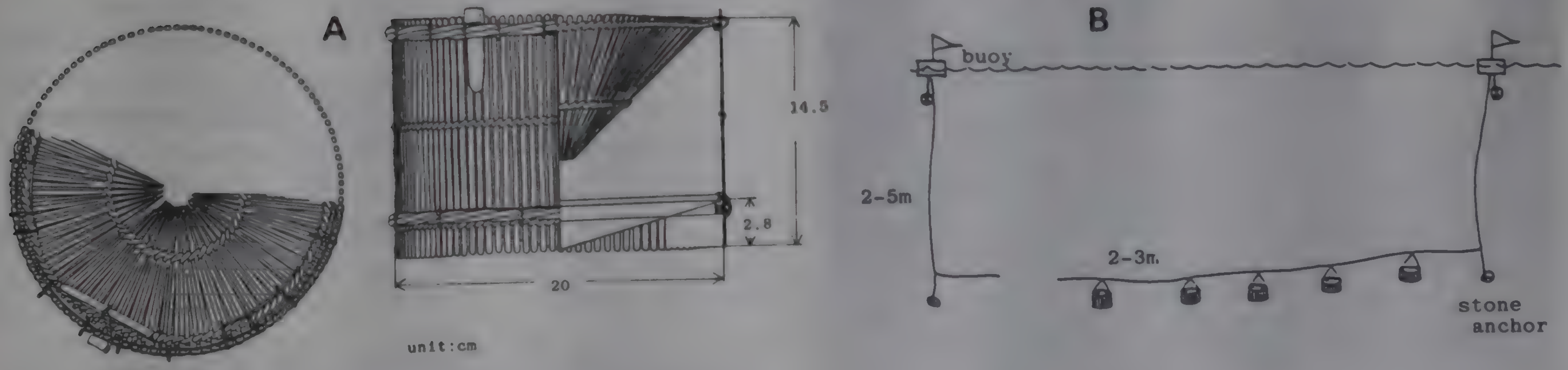


Fig. 1. Prawn potting operation: A. A prawn pot "Ebi-tatsube", made of split bamboo for catching oriental river prawn *Macrobrachium nippones* and striped prawn *Palaemon paucidens* in Lake Biwa, Japan. (Photo reproduced with permission from the Board of Education of Shiga Prefecture. B. A series of prawn pots in operation, 100 pots make one unit or set. The buoys are placed at each end of a set.

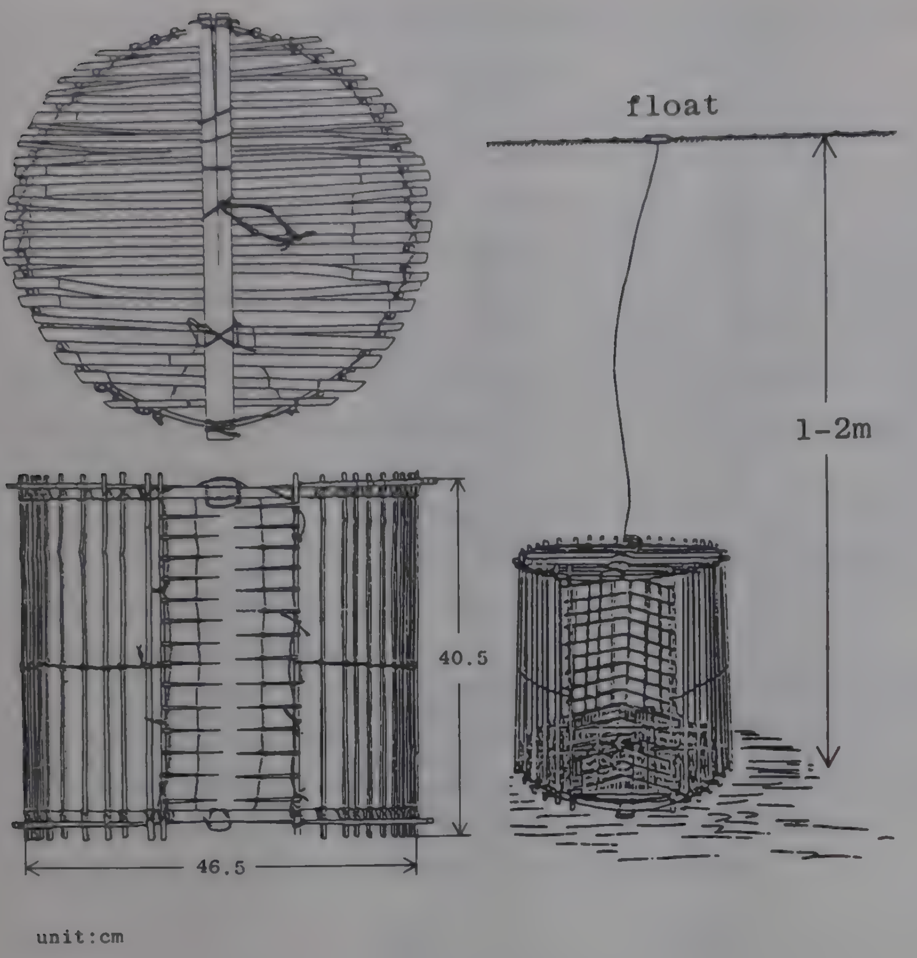
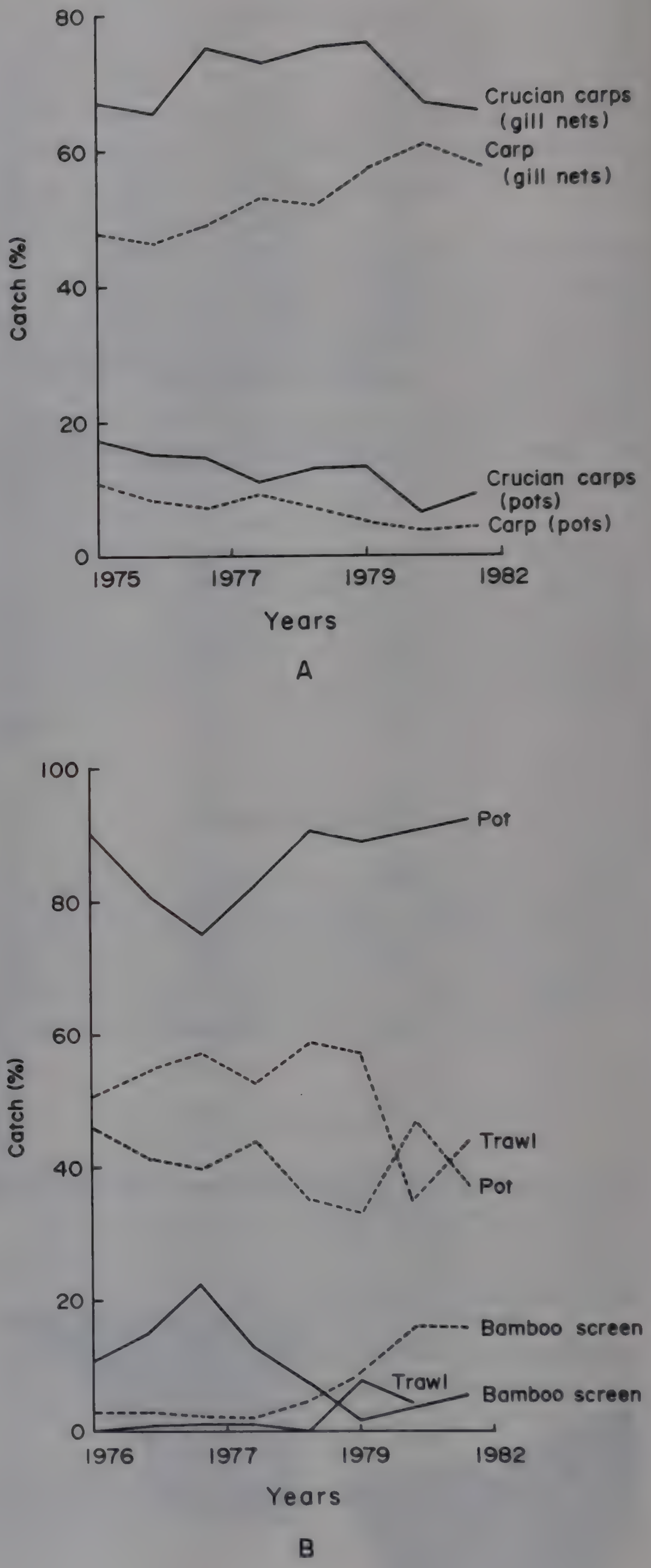


Fig. 2. Setting method of basket trap I: A basket trap I "Koi-tatsube" or "Funa-tatsube", made of bamboo split for catching carp *Cyprinus carpio* and crucian carps ***Carassius auratus grandoculis*** and ***Carassius auratus cuvieri***.



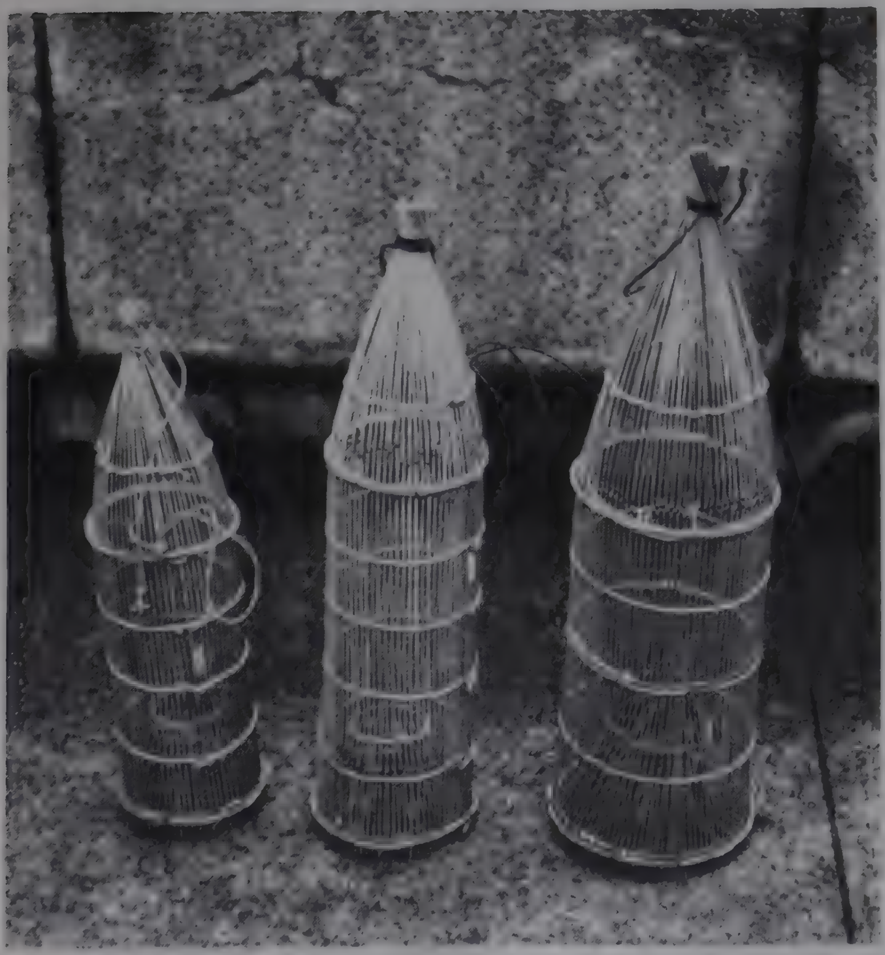


Fig. 3. Different sizes of basket trap II, "Ue", "Sen" or "Moji".

Fig. 4. The variations of percentage catch: A. Variations of percentage catch by type of fishing gear for fish. B. Variations of percentage catch by type of fishing gear for prawn. (————) represents the oriental river prawn, (– – – –) represents the striped prawn.

Fisheries Education in China

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Abstract

This study presents a general situation of the fisheries education in the People's Republic of China. It recalls the emergence of the vocational school of fisheries at the beginning of the century and the evolution of various institutions for training fisheries personnel of different categories. A general picture of all the universities, colleges and technical schools is given with their scope, enrollment and specializations. Spare-time schools, adult education and other organizations involved in fisheries training are also mentioned briefly. This paper also summarizes past experiences in fishery education in China and discusses prospects.

Historical Review

Fisheries education in China can be traced back to the turn of the century. During that period, China suffered severely from external imperialistic aggression, as well as internal feudalistic exploitation and oppression, and gradually changed into a semicolonial and semifeudal society. The people lived in destitution. Selfrenewal was impossible without developing the national economy and promoting education, two essential items in the Constitutional Reform and Modernization of 1898. Developing fishing enterprises meant exploiting marine resources, fortifying coastal defense and also advancing national economy and sea transportation.

A famous scholar and industrialist, Zhang Qian (also Zhang Jishi), 1853-1926, of Nantong, Jiangsu proposed to the Governor of Jiangsu Province and the Minister of Commerce to start a fishing corporation in 1904. The proposal was approved and the Jiang-Zhe Fisheries Company was set up at Wusong township by the confluence of Yangtze and Huangpu Rivers. Two vocational schools, fishery and maritime, were founded in the same year. The former was the earliest institution of fishery education in China. As it was affiliated to a private enterprise, its enrollment was very limited and of elementary level, mainly to supply workers for the company.

Two years later, another enterprise, the Zhili Fishery Co., was formed in the northern province in Tianjin. Being short of qualified personnel to manage modern fisheries, the company sent several investigation groups to Europe and Japan. In 1911, these groups founded the Study and Training School of Fisheries in Tianjin.

After the overthrow of the feudal reign of Qing dynasty in the revolution of 1911 led by Dr. Sun Yat-Sen, people were inspired to advocate education and to develop science and the economy. In the south, the Jiangsu Provincial Fisheries School was established at Wusong in 1912. In the same year, the Study and Training School of Fisheries in Tianjin was upgraded to Zhili Provincial Senior Vocational Fishery School. These two were the earliest full-time regular schools of specialized middle education under the auspices of provincial governments and were no longer affiliated to any enterprises.

These two schools were afforded with fishing ships for practice, experimental sites for aquaculture and workshops for aquatic products processing. In the first decade, foreign textbooks were used. From 1912 to 1922, the Jiangsu Provincial Fisheries School published a series of textbooks on the basis of their survey of fishery resources, fishing gears, navigation courses and main fishing grounds nearly all over the East China Sea.

With the development of a deep-sea fishery industry, middle education was upgraded to higher education in 1929. A two-year college course in deep-sea fishery was offered in Jiangsu Provincial Fishery School. Meanwhile, the Zhili Provincial Senior Vocational Fishery School in Tianjin was upgraded into Hebei Provincial Professional Fisheries School.

Before the outbreak of the war of resistance against Japan (1937-1945), fisheries education in China enjoyed a fairly rapid development. Middle technical schools emerged in every coastal province such as the Zhejiang Provincial Senior Vocational School of Fisheries at Dinghai County, Zhoushan Islands in 1915; in Xiamen, Amoy, financed solely by the famous overseas Chinese industrialist Chen Jiagen, the Jimei Schools of Fishery and Navigation were formed in 1920; the Yantai Study and Training School was established in 1923; in .Liaoning Province, a fishery school was set up at Yingkao in 1924; Guangdong Provincial Fishery School was founded at Shantou in 1935. Junior fishery schools and other training classes for fishermen also appeared in many towns in the early 1930s.

During the war, 1937-1945, most fishery schools suffered damages. The only remaining school was the Hechuan Fishery School in Sichuan Province, organized in 1941 for training technicians in freshwater pisciculture.

In 1945-1949, fishery schools were rebuilt successively in Shanghai, Zhejiang (Zhapu), Jiangsu (Chongming) and Hebei (Tianjin). Those in Fujian, Guangdong and Liaoning were restored at their former campuses. Up to 1949, there were two professional fisheries schools (Wusong and Hebei) and five senior vocational fisheries schools (Zhapu, Chongming, Dalian, Jimei and Shanwei) with a total enrollment of about 1,000.

The establishment of the People's Republic of China in 1949 brought about great achievements on all fronts. After rehabilitation and development, the annual fishery yield in 1952 tripled that of 1949. In 1952, several famous biologists went into fisheries education. The Wusong Professional Fisheries School and schools from Zhejiang and Jiangsu were amalgamated into the first higher educational institution named Shanghai Fisheries College (renamed Shanghai Fisheries University recently) which was multidisciplinary and national in character and was under the auspices of the National Fisheries Ministry.

Another college was founded at Zhoushan Islands in 1958 by the Zhejiang Province fisheries authority. In 1980, the Central Government upgraded the technical schools in Dalian and Zhanjiang to two colleges and set up a new one in Xiamen, Fujian. Departments of Fisheries were established in the following colleges: Shandong Oceanography College, Central China Agriculture College and Tianjin Agriculture College.

There are also middle technical schools distributed in the following provinces and municipalities: Liaoning, Heilongjiang, Hebei, Tianjin, Shandong, Jiangsu, Fujian, Hubei, Sichuan, Shanghai, Guangdong, Guangsi, Beijing and Zhejiang. Furthermore, some large enterprises have their own training classes. Academic societies of fisheries science sometimes offer special courses and correspondence schools. Middle technical schools in fishery areas offer fishery lessons or open vocational classes.

Present State

Generally, education in China is of two kinds: regular and adult.

Regular education is the principal means of cultivating scientific and technical personnel. There are four grade levels in regular fisheries education: the tasks of which are undertaken by the fisheries university, fisheries colleges and middle fisheries schools.

A university or college admits only the student who has graduated from an accredited middle school and passed an entrance examination. In most cases, a baccalaureate degree is granted after four years. Masteral and doctorate programs will be offered soon. In order to train more technicians in a shorter period, one fisheries university and four colleges in China concurrently offer two-year courses of senior-collegiate programs as well. Specializations include: Marine Fishing, Marine Engine Management and Fishery Economic Management.

The list of courses for the undergraduate program include: Fishery Resources; Marine Fishery; Freshwater Fishery; Freshwater Pisciculture (Aquaculture); Mariculture (Marine Farming); Fishery Machinery; Fishery Electronic Instrumentation; Fishing Vessel Designing and Engineering; Fishing Vessel Power Engineering; Fishing Harbor Designing Construction; Aquatic Products Processing Technology; Refrigeration Technology; Canned Food Technology; Food Engineering; Food Science; Fishery Economics and Management; Marine Engine Management.

The courses take four years, after which a student is granted a BFS or BFE degree and gets an appointment from the government.

In the Shanghai Fisheries University, postgraduates of MFS programs have five specializations: Ichthyology, Aquaculture, Marine Products Processing, Marine Fishery and Fish Ecological Environment.

Higher fisheries education has now a total enrollment of about 5,000.

Middle fisheries technical schools in China are under the provincial or municipal governments. They supply medium-grade technicians for fishery production and management. Students who have finished junior middle school are admitted to a three-year training in practical technique and basic scientific knowledge. After graduation, students are taken in by the local government.

There are 15 middle technical schools located along coastal cities. In addition, some agricultural schools in the inland provinces have also established fishery sections.

In such schools, different specializations such as Marine Fishing, Aquaculture, Marine Engine Management, Aquatic Products Preservation and Refrigeration are set up according to local requirements. Total enrollment in the middle fishery schools is about 7,000.

Adult education in fisheries is of the following types:

Fishery cadre training courses. Run by relevant colleges, institutes or administratives. They are further classified into: (i) those that cultivate personnel for a one-year schooling period and (ii) those that propagate some particular skills or technology for about a fortnight to three months at most. There is provision for all expenditures and students are paid salaries by their employers.

Evening schools. Affiliated to enterprises or institutions which offer two or three years of basic knowledge, as well as technical subjects for their workers.

Correspondence courses. Run by academic societies or other organizations.

Broadcasting or television programs. Sponsored by relevant fisheries institutions for the public, especially the fishermen, to spread new information and knowledge on fisheries in order to promote technological innovation.

Prospects

Fishery production has considerably developed since the founding of the People's Republic of China. In the last five years, total production increased by 60%. Although offshore fish resources exhibited a declining trend, the annual yield was steadily maintained. Aquaculture expanded rapidly; its yield in 1984 almost doubled that of 1980. Demand for more and highly qualified scientific and technical personnel has grown. Consequently, fisheries education in China must respond to this need.

The educational system is undergoing a national reformation. Every university, college or school aims to enlarge its enrollment; readjust the structure of courses and categories of schools; and improve its present system of education, teaching contents and teaching methods. It can be predicted that the number of middle technical schools will be greatly increased so as to gradually rationalize the personnel ratio of different grades. The number of colleges or universities will not sharply increase, but enrollment will certainly expand. In the inland provinces, some agriculture colleges will set up more departments of fisheries.

In a few years, fisheries education in China will make some significant progress in both quality and quantity.

Progress on the Fisheries Science Applications for Microcomputers

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Abstract

A microcomputer-based system for the analyses of small data sets of the sort encountered in fisheries stock assessment problems is presented. Applications software allowing over thirty-five kinds of analytical procedures were written in the Microsoft (TM) implementation of interpreted BASIC. A system architecture was formulated with the following characteristics: hardware transparency; a 'manual free' user interface; simple data entry and editing capability; a standard structure into which new or modified applications could be merged; a file management protocol consistent with proprietary spreadsheet software; and graphics options for appropriate procedures.

Introduction

This report describes a microcomputer-based system for solving numerical problems unique to fisheries science. At this time a manual, authored by S. Saila, C. Recksiek and M. Prager, which will include a system description, user's instructions and detailed examples of included procedures from the fisheries science literature is being prepared. This presentation will focus on design aspects of the Fisheries Science Applications System (FSAS).

The intent of the manual's authors was to build upon earlier work, principally that of Pauly (1984), in bringing analytical procedures from the literature into a computational environment where small data sets can be analyzed quickly without dependence upon mainframe computers and without specialized expertise in computer programming. Although many analytical processes from the life sciences have been translated into computer program code (review by Hensler 1984), a system designed for 'field' use by fisheries professionals was apparently lacking. However, the recent worldwide popularity of microcomputers, particularly of the IBM (TM) 'clone' types, indicated the high probability of acceptance of a system specifically designed for that hardware environment. Hence, the authors have brought a variety of specialized computational tasks into a system for the Microsoft (TM) implementation of interpreted BASIC (BASICA).

FSAS Features

The FSAS was designed according to several perceived criteria, the primary overall criterion being the provision of maximum computing power at minimum cost in the fisheries analysis setting:

1. Hardward transparency. In view of the popularity of the IBM Personal Computer (TM) and its many substitutes, it was decided to write program code which would run on the most fundamental of the currently marketed configurations. These days this amounts to a machine with two flexible diskette drives, graphics screen (an option with some systems but a 'stock' item with others), a printer, and a version of Microsoft's (TM) operating system (DOS) 2.1 or above. Today there are a variety of systems available, including the portables, which match these specifications.

Running the FSAS amounts to: (a) turning on the computer with the system diskette in the left-hand drive; (b) running system programs GRAPHICS and BASICA; (c) exchanging the system (DOS) diskette for a diskette containing the FSAS programs desired for the work session; and (d) loading (via the LOAD statement of BASICA) the FSAS primary control and file management program KEYSTONE.FSU and running it (via RUN). Experienced microcomputer users could depart from this straightforward procedure, e.g., by placing all the FSAS routings on a hard disk, but the 'ordinary configuration' will allow the system to run.

2. Ease of use. A major design criterion for the FSAS was minimizing dependence upon prior experience with computers and computer programming. To that end, a goal has been to create a 'manual free' user interface. This has been accomplished with all procedures being controlled by menus which direct the user in a step-by-step manner.

The system consists of a primary switching program and a collection of applications programs. Both are coded in the BASIC programming language, Microsoft's (TM) BASICA, in this case. The switching program, called KEYSTONE, contains most of the menus. It allows the user to create, edit, save and retrieve data sets. In addition, it helps the user select the application program of interest. Essentially KEYSTONE helps the user gain access to an applications program without much dependence upon a manual. The FSAS system KEYSTONE and applications programs are summarized in Table 1.

At this time the FSAS practically consists of microcomputer BASICA program files which fit onto four flexible diskettes (four sides). Each diskette holds KEYSTONE and FSAS applications programs. A user simply uses the diskette containing the applications program of interest. The user provides his own flexible data diskettes (or other medium of his choice such as a hard disk) which are initialized to receive data via the DOS FORMAT program.

3. Simple data entry and editing capability. The FSAS system operates on a single two-dimension array of numbers, e.g., a set of 25 fish lengths and weights (a 2 x 25 array). KEYSTONE's menus direct the user to a simple editor which allows entry or editing of the numbers in the array. The system includes protocols for dealing with missing values.

Once such array is defined, control is transferred to an applications program for analysis. This is done through BASICA's CHAIN and COMMON statements in the program code. The user is free to perform analyses and, afterward, 'return' to KEYSTONE for file management tasks, including directing control to other applications programs.

A useful feature of most FSAS applications programs is the property of being able to edit data while control remains within the applications program. This is done with a MINI-EDITOR which is similar to KEYSTONE's editor; indeed the editor subroutines are the same for KEYSTONE and those applications programs having the MINI-EDITOR. This is advantageous because a user may wish to change or remove a value and rerun an analysis. This feature allows doing it without switching control back to KEYSTONE. It is also useful because each applications program may be run by itself. That is, KEYSTONE is not really necessary to run most applications programs. For instance, if the user wished to find the mean of 20 numbers and view a histogram, he need only, after having implemented BASICA, insert the diskette containing UNISTAT into the left-hand drive, load and run the program and use the MINI-EDITOR to enter the data. After the data were correctly entered the user would execute the program which, in turn, would display the data set's statistics and, on option, a histogram.

At this point, it should be mentioned that some FSAS routines do not operate on a data set, e.g., B-H3 (Table 1). In these cases, the applications programs simply calculate values of a complicated formula; so, no data editing capability is included (but formula parameter entry and editing are).

One of the most important, and fairly unique, features of the FSAS applications programs operating on data sets is the provision of a sample, or test, data set embedded in the program code. This capability was engineered to enable the user to become acquainted with the performance and output of the applications program. Most test data sets are drawn from the literature. To use an applications program, the user would, ideally, after becoming familiar with the literature cited in the manual's program description, simply run the program and invoke the analysis using the internal test data set. By having the data set built in, the user is free to concentrate on the analysis and its results, and not on the details of data set input. Some users, if using a data set the same size or smaller than the test set, could simply edit in their own data onto the test data with the MINI-EDITOR and rerun the analysis. Control change to KEYSTONE for file management remains possible at any time.

4. Standard code structure. Each applications program is coded in standardized sections or modules. There are three types of programs in the FSAS which, for convenience, are termed SHELL 1, SHELL 2 and SHELL 3 programs (Table 2). SHELL 1 and SHELL 3 programs operate on data sets, while SHELL 2 programs evaluate formulas, as discussed earlier. SHELL 3 programs have no MINI-EDITOR to save working space. The FSAS system at this time has only one SHELL 3 program, FISHPARM (Table 1). Since FISHPARM applications code is so extensive, there would be little room for the MINI-EDITOR subroutines due to the 64-kilobyte restriction to combined program and variable space in the BASICA environment. The SHELL 3 programs, FISHPARM being the only case, need KEYSTONE to be operational, but SHELL 1 programs may be run with or without KEYSTONE. The latter is also true of SHELL 2 programs.

So, in translating a particular procedure from the literature into FSAS code, the appropriate shell modules consisting mostly of original system subroutines have been merged with a code unique to that procedure. Individuals having programming experience may find it useful to modify existing or create a new applications program code because it is reasonably easy to locate in the program listings.

5. File management protocol. Data set storage and retrieval as flexible diskette (or hard disk) files is accomplished only by KEYSTONE. Files are written in the standard Data Interchange Format (DIF). DIF files may be read or written by a variety of other software, particularly some of the recently developed proprietary spreadsheets. Therefore, a person acquainted with the use of a spreadsheet, e.g., Lotus 123 (TM), may use the latter's powerful data manipulation capabilities to create DIF files readable by the FSAS. In practice, a spreadsheet would be used to maintain a database and to create data subsets consistent with the requirements of a desired FSAS applications program analysis.

6. FSAS graphics. Several FSAS applications programs include graphics screen displays. For instance, program POWER which calculates parameters of the allometric equation, such as that which describes a length-weight relationship, permits the user to display the data as a scatter plot onto which the derived function is graphed. Likewise UNISTAT, which computes univariate statistics of grouped and ungrouped data sets, includes an optional histogram display. Other application programs do not include graphics when they are not really useful, e.g., JOLLY, a population parameter estimation program operating upon tag-recapture data, includes no graphics capability.

It should be mentioned that the FSAS SHELL 3 program FISHPARM includes graphics capability which portrays bivariate data and derived functions but the code is in a separate program, transparent to the user, which is accessed via BASICA's CHAIN and COMMON statements. Again, this design is motivated by the desire to save working space.

Incidentally, FSAS graphics subroutines allow the user to adjust the range over which data are displayed. Additionally, the user may save a hard copy of the screen display by having run the DOS system program GRAPHICS before BASICA during system startup.

Discussion

Perhaps the principal advantageous features of the FSAS are the simple data and parameter editors and, in the case of the SHELL 1 programs, the inclusion of test data sets. It is hoped that by using DIF files, the power of the proprietary spreadsheets may be used to enhance the capabilities of the FSAS. An additional advantage is that some of the analytical routines are based upon direct nonlinear estimation procedures which facilitate error estimation.

One of the possible disadvantages that has become more fully apparent during design and development of the system is the 64-kilobyte restriction of BASICA. This has certainly influenced many system design choices. Without this restriction there would be no need for SHELL 3 type programs since all the editor and graphics routines could otherwise have been included in one program. Indeed, the file management code of KEYSTONE could potentially be included as well. Also, interpreted BASIC programs doing a lot of 'number crunching' tend to be slow. As a result, future plans call for creating a compiled version of the FSAS so as to take advantage of larger memories readily available today at modest cost and to enhance computing speed.

In conclusion, the FSAS is clearly useful in the analysis of 'small' data sets often encountered in fisheries science and in a field-laboratory setting. It is hoped that the system architecture is easy enough to use so that the investigator may be free to concentrate on the application at hand and not be greatly concerned with running the computer.

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Table 1. Fisheries Science Application System (FSAS) BASICA program file names with program procedures.

KEYSTONE	Primary control and file management
FISHPARM	Non-linear least squares parameter estimation for selected fisheries models
UNISTAT	Computation of univariate statistics for grouped and ungrouped data sets
BIREG	Bivariate regression analyses
BAKCAL	Back-calculation of length at age via Fraser-Lee formula
POWER	Power relationship calculated by ordinary least squares on log-transformed data with bias correction
PRINCOMP	Finding a 95% equal frequency ellipse from principal components analysis
RAFAIL	von Bertalanffy growth equation
RICKER	Ricker's yield model
LCOH	Jones' length related cohort analysis
MARTEN	Calculating mortality rates and optimum yields from length samples
BARTTR	Bartlett's test for homogeneity of variance including four transformations
PROD 2	Cohort production estimates with variance
SINTAG	Weighted regression method for survival rates based on a single tagging experiment with continuous recaptures
SHEP	Length composition analysis using von Bertalanffy growth parameters
LP	Calculation of total allowable catch
HILLIS	Assessing the rate of discarding commercial species when the total weight of discards is known
ZIPPIN	Population estimation using removal-depletion
FABGROW	von Bertalanffy growth equation estimated by Fabens' method for tagging data
MESH 1	Effects of gear mesh size on catches and immediate and long-term changes in catch
MESH 2	Calculating yields by species and mesh size
LOGIT	Calculation of LD_{50} values, mesh selection, and percent maturity
OPTAGE	One variable minimization method to compute optimum age and size
B-H3	Beverton-Holt YPR equation with constant parameters and yield maximization varying both F and age at first capture
TBH	Beverton-Holt YPR equation with variable (negative exponential) natural mortality
SEGREG	Segmented regression analysis
LOGSER	Estimation of log series parameters
IVALUE	Calculating the value of a similarity matrix
CLUTES	Testing clusters for significant differences (using IVALUE output)
KTEST	Test statistic on K parameter of a negative binomial distribution (K is related to species abundance)
NETMED	Computation of confidence limits of medians for a series of net catches
RECRUIT	Recruitment calculation by Beverton-Holt's method
JOLLY	Population parameter estimation from mark and recapture using Jolly's method
SCHNUTE 1	Improved stock production model

Table 2. Properties of the FSAS applications programs. The "+" indicates an included capability; the "–" indicates that a capability is lacking.

	Applications program types		
	Shell 1	Shell 2	Shell 3
Operates on a data set	+	–	+
Includes the MINI-EDITOR	+	–	–
Includes a test data set	+	–	–
Ability to be fully functional without KEYSTONE	+	+	–

Indigenous Fishing Crafts in Java, Indonesia

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Abstract

In 1984, a survey was conducted in eight coastal villages around Java Island on 175 fishing crafts by making 45 sectional measurements and 20 craft characteristic entries plus their detailed hull-line drawings. The representative crafts of each village surveyed are described according to their hulls, such as dugouts, semidugouts and planked hulls. The hulls of Java crafts as described have distinctive local variations. Statistical considerations including naval architectural data were examined by using discriminant and cluster analysis to compare the Java craft hull type with similar fishing craft in Japan, Korea, China and Thailand. The results show that the hull type of the Java planked crafts was developed independently using the local dugouts and semidugouts as the basic design.

Introduction

In 1981, 95% of Indonesian fishing crafts were of 10 gross tons (GT) or less; 98% of marine fish landings came from these crafts operating in coastal waters (Indonesian Directorate General of Fisheries 1981). Accordingly, the indigenous fishing crafts in coastal waters have a very important contribution to Indonesian fisheries. However, the motorization of fishing crafts has been accelerated since the declaration of the 200-mile Exclusive Economic Zone (Anon. 1982). Motorized crafts may displace the indigenous ones. The survey was carried out to record the current state of the indigenous fishing crafts in eight coastal villages around Java from July to August 1984, especially the hull-type crafts and their fishing and to consider their future modernization in comparison with those of other countries around Indonesia.

Methodology

Fishing crafts and gears were catalogued and inventoried at the beginning of the survey.

A manual for the survey was made for uniformity in recording the complex data. A special measuring comb consisting of movable teeth of wooden strips 900 x 15 x 2 mm and a framework of 700 x 800 mm size was made for the hull measurements.

The survey was divided into four stages: (1) recording general descriptions of the village; (2) interviews of fishermen by questionnaire; (3) measurements of 25 dimensional parts of around 20 crafts in each village; and (4) detailed measurements of hull lines on several crafts by village.

The fundamental statistics consisted of frequency distributions of data, vectors of means and standard deviations, and figures of interrelations between crafts by various aspects. Cluster analysis was then applied with various dimensional data for comparing local differences among the crafts around Java.

The CPU programs on the above analysis were from Tanaka et al. (1984). Various numerical characteristics of the naval architectural data were calculated by means of a hydrocalculation program in BASIC, coded by Jyanuma (unpublished data) for a microcomputer, PC-9801.

Results and Discussion

The location of the fishing villages and the fishing crafts surveyed are shown in Fig. 1. The 15 indigenous fishing crafts found around Java have various types of construction, sail, outrigger and local characteristics. Among them a dugout with a single-hold outrigger in Rembang was quite different from the others.

Statistics on sail, drive engine, fishing gear, fish caught and hull material of planked craft are shown in Fig. 2.

There are three kinds of hulls amongst the crafts, i.e., dugout, semidugout and planked craft. The principal types around Java are shown in Table 1. Generally, the planked type is the largest among the three and the dugout and the semidugout are comparable in size. The semidugout is very narrow in beam with a 51 cm mean (range 21-88 cm).

A series of comparisons was made of the dimensions of the principal Java types, i.e., length overall (Loa), beam

(B), and depth (D), with those of 58 Thai crafts (RACIC 1967).

The distribution of Loa and B is given in the following regressions:

Java planked crafts (N=113, r=0.8382)
$B = 0.222864\ Loa + 18.9378$
Thai planked crafts (N=58, r=0.8021)
$B = 0.257358\ Loa - 22.7507$
Java semidugouts (N=43, r=0.5283)
$B = 0.067253\ Loa + 3.14267$

The correlations between them were significant at the tolerance level of 5% on the three kinds of crafts, but not the Java dugouts (r = 0.24). The linear regression lines for the Java and Thai planked crafts resemble each other; however, data on semidugouts were quite independent with narrower beam than the other two. Data on dugouts were scattered between the two kinds of planked crafts and the semidugouts.

Significant correlations between B and D were not found between Java dugout and semidugout, as the data were concentrated within a narrow range in size. On the other hand, significant linear regressions at the tolerance level of 5% were calculated for the planked crafts in Java and Thailand:

Java planked crafts (N=113, r=0.8553)
$D = 0.284896\ B + 15.7797$
Thai planked crafts (N=58, r=0.7790)
$D = 0.320658\ B + 33.1318$

To compare the 113 planked crafts, cluster analysis applied with the group average method and Mahalanobis distance was made on Loa, B, D (in meters), volume LBD (in cubic meters), and the ratios L/B, L/D and B/D (Fig. 3).

As shown in this dendrogram, the clustering is generally not so clear because of the narrow range in Mahalanobis distance. The planked crafts were mainly of two major groups, G1 and G2. The G1 of clusters I-IV was a group of smaller and intermediate crafts and G2 of clusters V-VII was a group of bigger crafts. Thus, the planked crafts were widely scattered into seven clusters including a cluster of smaller and shallow crafts.

The frequency distribution of crafts by cluster and village or fishing gear (Fig. 3) showed that all crafts from Tuban, Rembang and Cilacap were classified as G1. These smaller crafts used hand line, cast net, gill net, trammel net and long line while the bigger crafts (G2) used purse seine, Danish seine and gill net in Cirebon, Pasuruan and Pelabuhan Ratu.

As shown in mean vectors, the G1 were classified into cluster-I (CL-I) of fine but relatively deeper hull, the CL-II of the smallest but rather fair hull, the CL-III of the biggest but rather deep hull and the CL-IV of fair and deepest hull. The three clusters of G2 might be separated into shallow, deep and deeper.

A comparison of various fishing crafts by three hull types from the results of hydrocalculations on load condition of crafts in various countries, i.e., Europe (Smyth 1929), Korea (Korea, Government General Fishery Experimental Station 1929), China (Takayama 1938) and Japan (Shibata et al. 1976) is given in Table 2.

In this table, d is a draft when loaded condition and D/W is the displacement in tons. HB/Loa is the position of the longitudinal center of buoyancy in relation to midships, expressed as a ratio; the minus value (-) indicates a position forward of midships. Thus, if a minus value, the immersed hull body is relatively bigger in forward of midships. KB is the distance of center of buoyancy above the bottom of the keel. KB/d is ideally 0.5 for a box-type craft and 0.67 for a craft that is triangle in cross section. KM is an indicator of static stability of a craft.

The KM values in Table 2 show the higher stability of European sailing crafts, while the Japanese semidugout, Sabani in Okinawa and the river planked crafts in Sumatra had relatively low stability.

Acknowledgement

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Table 1. Principal dimensions of indigenous fishing crafts around Java in 1984 (cm).

		Loa				B				D			
	N	mean	S.D.	min	max	mean	S.D.	min	max	mean	S.D.	min	max
Dugout	19	757	73.3	604	917	83	10.6	61	107	54	9.4	43	73
Semidugout	43	717	106.4	475	897	51	13.6	21	88	58	7.4	43	73
Planked	113	801	214.5	422	1,280	197	57.0	100	345	72	19.0	30	122

Table 2. Mean principal dimensions (cm) and naval architectural data of various crafts in various countries.

	N	Loa	B	D	d	D/W	Cb	Cp	Cw	Cm	HB/Loa	KB/d	KM/B
Dugout	11	492	69	35	19	0.44	0.45	0.62	0.65	0.69	0.03	0.60	0.47
Semidugout													
Java	4	662	56	49	25	0.52	0.53	0.67	0.76	0.79	0.83	0.60	0.52
Japan	5	662	111	42	22	0.79	0.38	0.57	0.49	0.61	−2.59	0.61	0.39
Planked													
Java–EC	9	560	142	52	26	0.84	0.38	0.57	0.55	0.67	0.31	0.59	0.47
–W	7	935	262	85	42	4.78	0.38	0.56	0.54	0.67	1.04	0.56	0.50
Sumatra	3	473	143	50	25	0.53	0.34	0.63	0.54	0.67	−1.80	0.64	0.39
Europe	9	1,135	384	211	118	21.73	0.34	0.60	0.63	0.57	3.09	0.65	0.58
China	10	1,285	326	103	62	24.00	0.43	0.58	0.59	0.75	5.48	0.58	0.48
Korea	11	836	273	100	53	7.33	0.45	0.64	0.66	0.71	6.05	0.59	0.56
Japan–N	17	573	157	51	36	2.34	0.49	0.67	0.67	0.73	0.68	0.63	0.48
–W	9	550	143	51	37	1.35	0.44	0.63	0.67	0.65	9.12	0.61	0.48

Note: Java–EC and –W are East, Central and West Java, and Japan–N and –W are Northeastern area of Honshu and Nagasaki Prefecture in Japan.

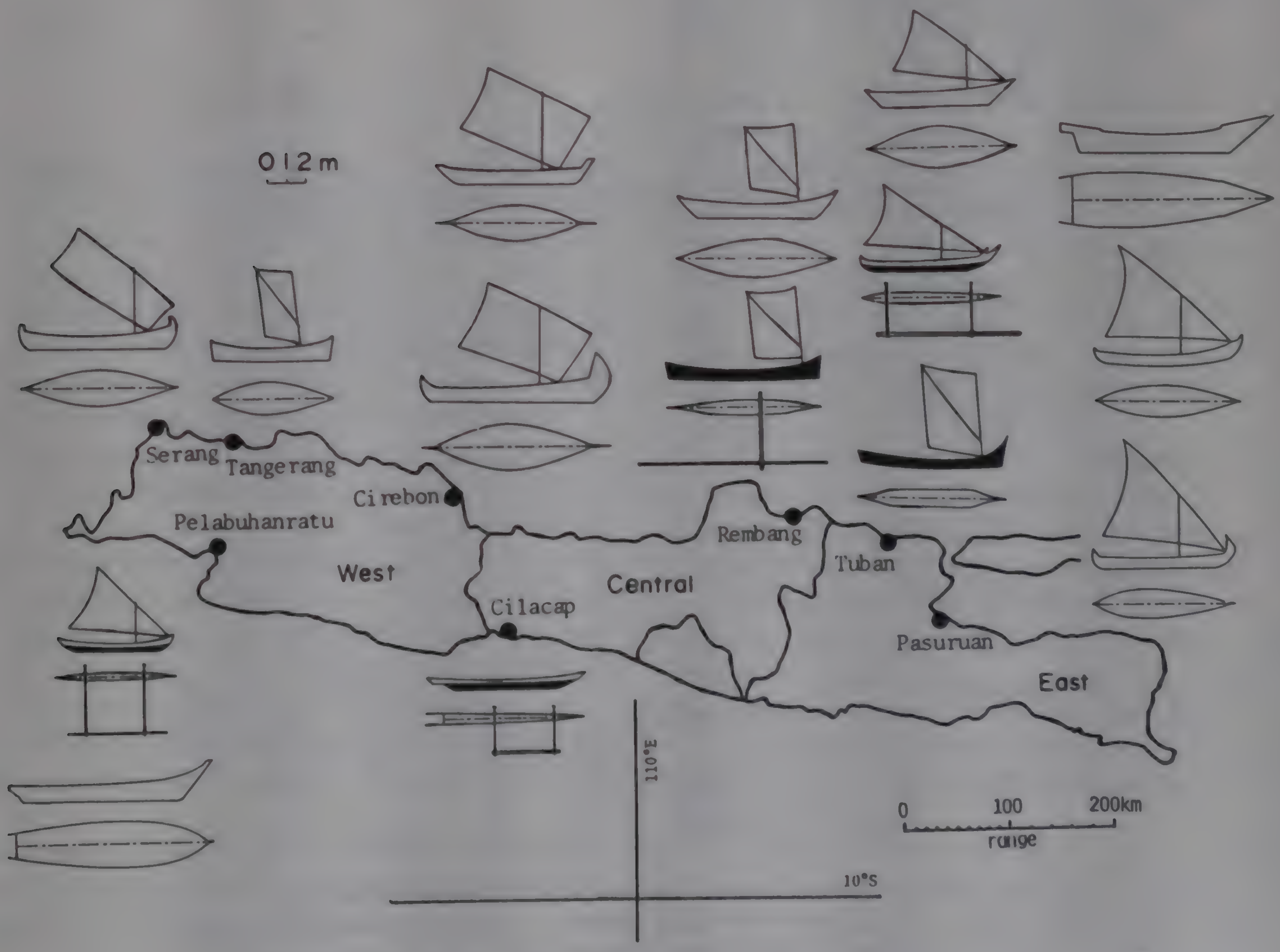


Fig. 1. Various fishing crafts around Java in 1984. Black spots are survey stations.

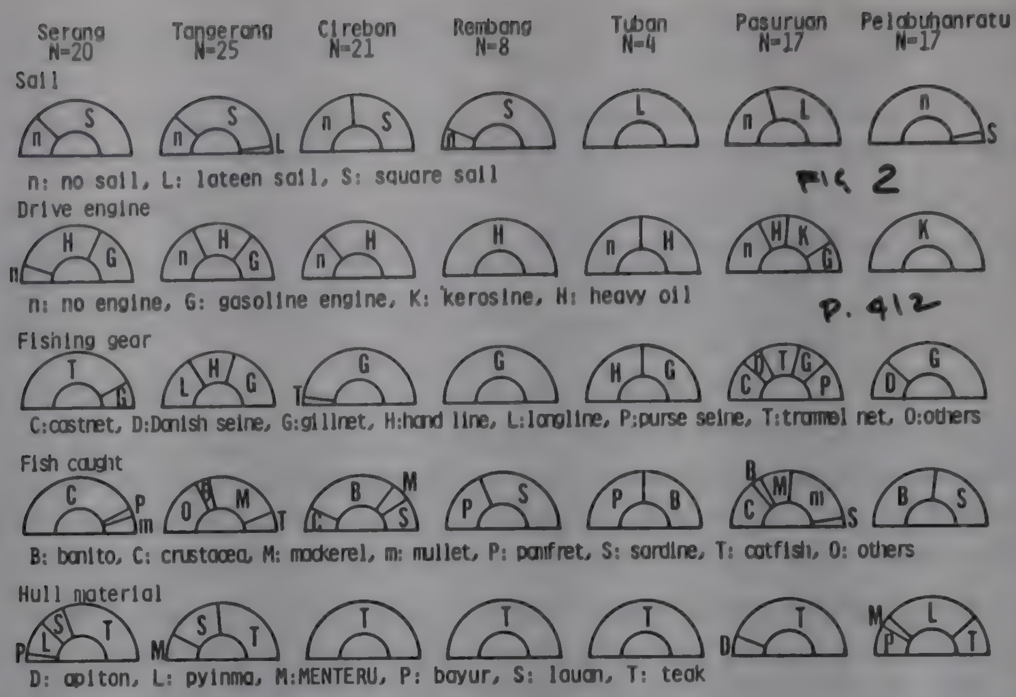
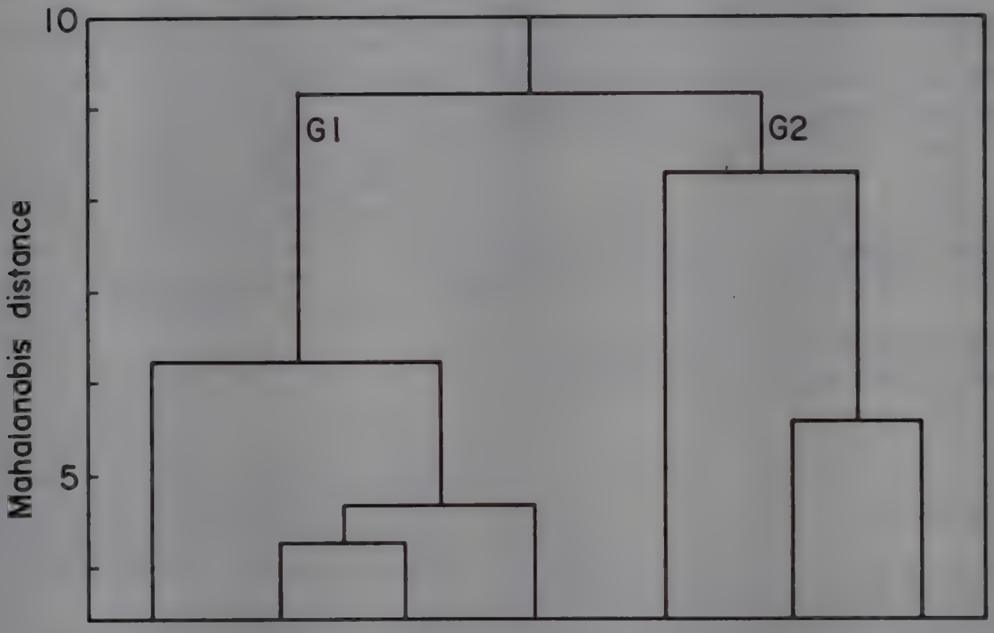


FIG 2

P. 412

Fig. 2. Frequency distributions on various catalogous data of Java planked crafts by fishing village in 1984.



Cluster	I	II	III	IV	V	VI	VII
N	24	49	10	5	3	17	2
Village							
Serang	9	11	0	0	0	0	0
Tangerang	0	19	0	5	0	0	1
Cireban	7	0	3	0	1	10	0
Rembang	0	7	0	0	0	0	1
Tuban	0	2	0	0	0	0	0
Pasuruan	8	2	1	0	2	3	1
P. Ratu	0	7	6	0	0	4	0
Cilacap	0	1	0	0	0	0	0
Fishing gear							
gill-net	9	29	9	0	1	10	1
tramel-net	14	9	0	0	0	0	0
hand-line	0	2	0	5	0	0	0
long-line	0	7	0	0	0	0	1
Danish-seine	0	0	0	0	1	5	0
purse-seine	0	0	1	0	1	2	0
cast-net	1	2	0	0	0	0	1
Mean vector							
Loa	7.74	6.66	9.80	7.13	11.03	11.24	8.51
B	1.60	1.69	2.19	2.33	2.90	2.88	2.95
D	0.57	0.65	0.94	0.91	0.73	1.00	0.83
LBD	7.85	7.79	20.46	15.07	23.42	32.93	20.81
L/B	4.87	3.93	4.51	3.06	3.80	3.93	2.90
L/D	13.63	10.21	10.40	7.89	15.18	11.32	10.26
B/D	2.81	2.61	2.32	2.59	3.99	2.88	3.55

Fig. 3. Cluster analysis on principals and proportionals of planked crafts in Java. Top: cluster dendrogram, middle: frequency distributions of crafts by village or fishing gear, bottom: mean vectors of various data used for the cluster analysis. See text for abbreviations.

Traditional Inland Fishing Methods in Rajasthan, India

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Abstract

On account of the highly diverse nature of inland water areas of Rajasthan State of India, methods ranging from catching fish by hand to the operation of large and indigenously-designed nets are adopted for fishing. This paper describes the various fishing methods used in the area along with the constructional designs of various types of fishing nets and their mode of operation.

Introduction

Knowledge of Indians concerning fish and fisheries is quite ancient (Hora 1951). Paradoxical as it might seem, the state of Rajasthan in India with its sprawling Thar Desert is fairly rich in fishery resources. A variety of habitats, e.g., mountains, plains and desert, contribute to the hill-stream, riverine, lacustrine and reservoir fisheries. Thus, fishing implements also vary in accordance with the hydrographical conditions in different areas of the state.

There is a paucity of literature on the inland fishing methods and gear. Hornell (1924) described the fishing methods of the river Ganga. Hora (1926) dealt with a fishing implement from Punjab. Faruqui and Sahai (1943) gave the methods of catching fish in the state of Uttar Pradesh. Saxena (1964) dealt with the fishing nets and traps of the river Ganga near Allahabad. George (1971) had an account of the fishing gear and methods of India. Sehgal (1973) and Tandon and Sharma (1984) described the fisheries and the fishing methods of Himachal Pradesh state.

An attempt has been made in this paper to describe and classify the various fishing methods with related gears traditionally used in the state of Rajasthan, India.

Classification of Fishing Methods

Attempts to classify fishing methods and gears were made by Burdon (1951), von Brandt (1964) and Tandon and Sharma (1984).

Based on the above the inland fishing methods of Rajasthan can be of the following five types: (1) disabling type, (2) traps and barriers, (3) filter type, (4) entangling type and (5) self-fishing type.

DISABLING TYPE

The main aim of this type is to render the fish incapable of swimming. The following are disabling types:

Rod and line. This is one of the simplest methods of fishing. It essentially consists of a rod, a nylon twine and a hook. The hook is suitably baited to lure the fish and is kept in proper position by attaching a spoon or a stone. The most commonly-used baits are earthworm, wheat flour paste, small fish, frogs and insects.

Long line. This is in fact a cross line. It essentially consists of a main line with several pendant vertical lines called 'snoods' bearing hooks at equal distances. The ends of the main line are tied to wooden pegs one on each side of the river or the long line may be shot from a slow moving boat. It is kept in position by lead sinkers. In muddy or rocky bottoms, it is held off the ground by floats. The hooks are baited with bread, earthworm, wheat flour paste, small fish or frogs. Long lines are operated easily and are dropped into places where fishes are expected to be in some concentration.

Poisoning. The fish is disabled by the use of chemical powders or ground plant tissues. The result is indiscriminate killing of fish, which is illegal. The most commonly-used fish poison is rotenone obtained from the roots of the plants *Derris elliptica, D. uligmosa* and *D. lagensis*. However, fruit of bitter temru *(Diospyros cordifolia* Roxb.*)* found extensively distributed in Rajasthan is also generally used to stun fishes.

Dynamiting. Fish are paralyzed by underwater explosion. This method also results in mass mortality of fish and is illegal.

Spearing. The hunter sits on the river bank with a spear ready in his hand. The spear consists of a bamboo staff 1.8-2.1 m long to which is attached a sharp, pointed and barbed instrument of steel. As soon as a big fish surfaces the hunter aims at it and throws the spear to stab it.

Gunning. The man with a loaded gun sits on a stout branch of a tree or a cliff overlooking the river. As soon as a big fish comes near the surface of the water the hunter takes aim and fires.

TRAPS AND BARRIERS

Pot method. This is a primitive method of catching small fish. It consists of an earthen pot with a wide mouth. Some stone pieces or pebbles are placed inside the pot to make it heavy. A bait of wheat flour paste is also put inside. The mouth of the pot is then covered with a piece of cloth in which a hole is cut. Now the pot is fixed in the flowing water at a convenient place. Fishes tempted by the bait enter through the hole and thus get trapped. Small fishes like *Barilius*, *Danio* and *Puntius* spp. are caught by this method.

Bundh method. In this method a bundh or barrier is put in the channel, drain or rivulet to stop its flow. A small outlet is then made to divert water. The outlet is covered by a gill net or wire gauze to stop the escape of fish. The fishes are thus trapped in the gill net or picked up from underneath the stones.

FILTER TYPE

Drag net. This net is extensively used in rivers since it gives maximum catch and is easy to operate. Five to ten pieces are connected to one another to cover a large area of water. Each net piece is 7-10 m long and 4-8 m wide with a mesh size of 10-20 cm. The nets are bordered by a head line studded with floats of wooden reeds or synthetic balls and a foot line provided with sinkers of iron or lead. The head and foot lines keep the net in position.

Different drag nets are used according to the condition of the river. *Chhanta* is employed where the river is narrow. It is a drag net having single or double row of pockets at the bottom. *Mahajal* and *Chondhi* are used by fishermen brought by fish contractors from Varanasi and Gorakhpur districts of Uttar Pradesh. The net is spread out from the river bank in a semicircle, then both ends are dragged from the bank. All the major carps and catfishes are caught by this net.

Ghasita Jal. This net (Fig. 1) is used in the Jaisammand lake of Udaipur. It is a beach seine with one or more rows of peripheral pockets or folds towards the lower periphery of the net in which the fishes are collected. The pockets are formed by folding the lower periphery of the webbing along with the foot rope and sinkers and in turn secured at regular intervals. The mouths of the pockets are provided with sinkers.

ENTANGLING TYPE

Gill net. These are rectangular nets of a single wall made of fine nylon or cotton with a head rope but with or without foot rope. The size of the mesh and the height of the net varies from place to place. Floats made from scirpus stems keep the net afloat. The net is laid at night by a plank-built canoe in the manner of a stretched wall. It is hauled up the next morning and the fishes are caught in the meshes by the thickness of abdomen rather than by the head.

The gill net used in the Jaisamand Lake of Udaipur is locally called *Phasula.* It is a set net type and is made of terylene. There are 300 meshes in length and 20 meshes in depth with a mesh bar of 70 mm. The head rope is made of cotton 4 mm in diameter and 18.85 m in length. The fishing height of the net is 2.49 m. The floats are made of reed and number about 16-17 per piece. Tin cans, 30-40 in number, serve as buoys with a cotton buoy rope 4 mm in diameter and 0.5 to 0.8 m in length. The sinkers when used are of granite stone. Two sinkers, each weighing about 100 g are used per piece. The anchor is also made of granite stone weighing 10-15 kg. The anchor rope is made of hemp of 5-6 mm diameter and 15-16 m length. Thus 30-40 pieces of net are joined together to make the total length of the whole fleet about 565-574 m. This net is used in the Jaisamand Lake at depths of 5-16 m generally from November to January with a haul of *Catla catla, Labeo rohita, L. calbasu, Cirrhina mrigala, Tor tor, Silonia silondia, Mystus* spp. and *Wallago attu.*

Coverpot or plunge basket. These gears are cast on the fish which are collected from the top. They are usually made of wicker with the main opening at the top. *Tapar* (Fig. 2) used in the Jaisamand lake of Udaipur has the shape of a bell. The material used is bamboo strips secured by coir rope. Fishes generally caught with this type are *Channa* spp.

Cast net. This is a light bell-shaped circular net and weighted around the perimeter (Fig. 3). The mesh size and the maximum diameter of the net varies from place to place. There are two types of cast nets in use. These are the stringless cast net and the stringed cast net. The stringless cast net is more primitive than the stringed one. The stringless net is a simple piece of netting circular in form with a strong cord running through the peripheral meshes of the net with iron and lead sinkers. The net is skillfully thrown by a fisherman on the water so that it lands horizontally and encloses the fish as it sinks. When the net is raised or pulled up the circumference is reduced inward to form a pouch around the edge of the net to prevent the escape of the fish.

In Jaisamand Lake the cast nets used are with the closing string and without peripheral pockets. In these nets fishes are collected within a cavity of the net towards the lower portion while some of the fishes are gilled and entangled in the meshes. Three varieties of such nets locally called *Dhaiya, Bariya* and *Kathariya* are used in the Udaipur region. *Dhaiya* is made of cotton 20/5/2; mesh bar, 31 mm; height of net 2.9 m; peripheral circumference, 12.2 m; number of closing strings, 12. Fish generally caught are carps, *Mystus seenghala* and *Channa* spp. *Bariya* is made of cotton 20/3/2; mesh bar, 17.5 mm; height of net, 3.1 m; peripheral circumference, 11.6 m; number of closing strings, 12. Fish caught are small varieties of carps, catfishes and *Channa* spp. *Kathariya* is made of cotton 20/4/2; mesh bar, 7.5 mm; height of net, 3.3 m; peripheral circumference, 16.0 m; number of closing strings, 12. Fish caught are fry of major carps.

SELF-FISHING TYPE

This fishing method is dependent upon the physiological reactions of the fish to various chemical or physical stimuli. For example, light has been used from time immemorial to lure fish. Various sources of light have been used for the purpose. However, this method is illegal.

Discussion

A perusal of the fishing gear and methods traditionally used in Rajasthan, India, reveals that certain broad demarcations can be made regarding fishing gear of different water bodies. The topography of the area and the habit of the fish play a dominant role in the distributive use of fishing gear.

In the ponds, cast nets, coverpots or plunge basket, gill nets, traps and hand lines are used in that order of importance. The shallow depth of the ponds and tanks make the above gears more effective.

The various cast nets described by von Brandt (1964) are not found in India. However, he considers the cast nets originating from India. The stringless cast net is considered more primitive than the stringed one. While the coverpot or the plunge basket is limited to a certain depth, the cast nets are operated in relatively deeper water.

The design of gill nets varies from place to place. Some of the salient features are the absence of the foot rope, mesh size and height of the net. For example, the lacustrine gill net is higher than the riverine. However, the design in its turn affects the mode of operation of gill nets at various places.

In the lakes and reservoirs, the gill nets of the set type are the principal fishing gear because many underwater obstructions are encountered there. Beach seines are operated to a limited extent in areas where the bottom is clear such as Jaisamand Lake. In clear waters hand lines and long lines are not so efficient.

The fishing gear of the river system can also be divided into three sections: upper, middle and lower. In the upper reaches, where normally a swift current prevails, cast nets, coverpots and traps are common. In the pools, lines and gill nets of short depth are used.

In the middle reaches of the river, seine nets and gill nets are quite effective. Small meshed nets and noose are reported (Hora 1926) as suitable. Cast nets and barriers are of secondary importance.

All the beach seines like the *Maha jal, Chhanta jal* and *Ghasita jal* are operated in an identical manner from one boat. The net is payed in the form of an arc from the shore. The number of men required depends on the size of the net. However, the operational details of the *Ghasita jal* and *Chhanta jal* differ from other beach seines in that the head and the foot rope are not manipulated to concentrate the catch in the center unlike other beach seines. Occasionally the nets are operated as a drag net by paying the net parallel to the lake shore and dragging it from there.

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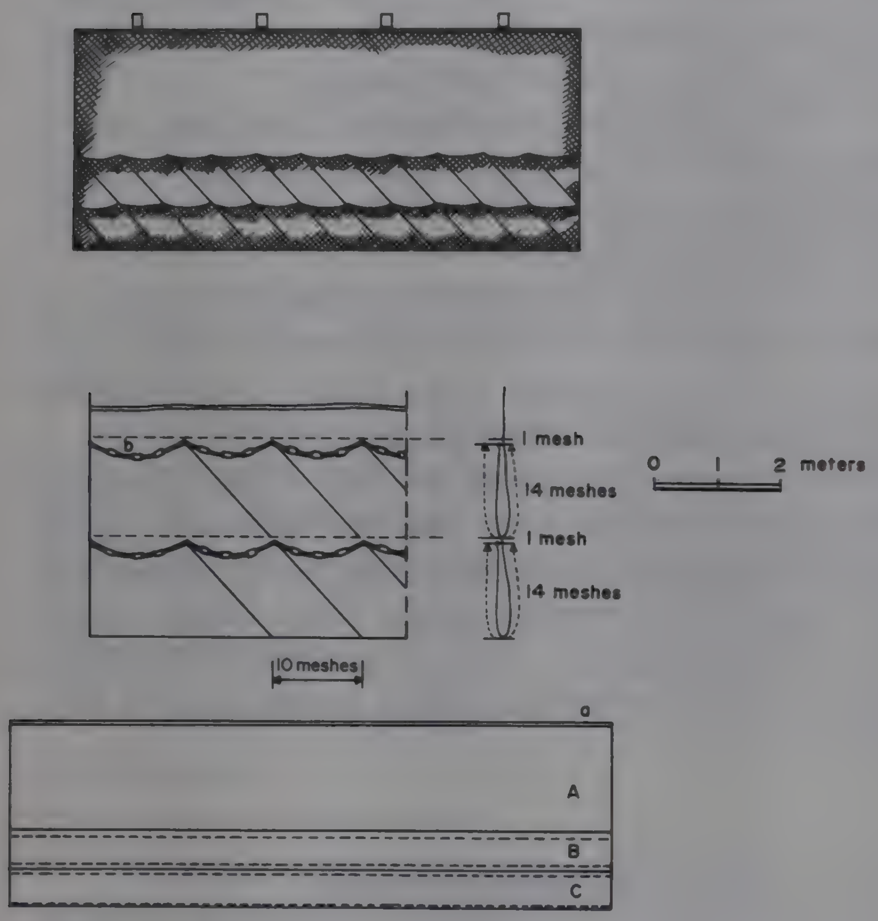

Fig. 1. Ghasita Jal.

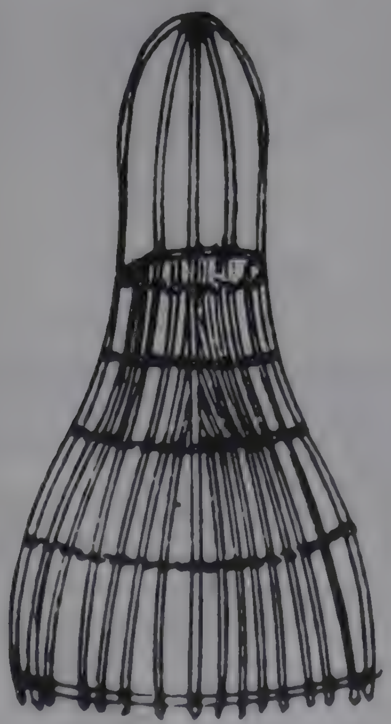

Fig. 2. Tapar

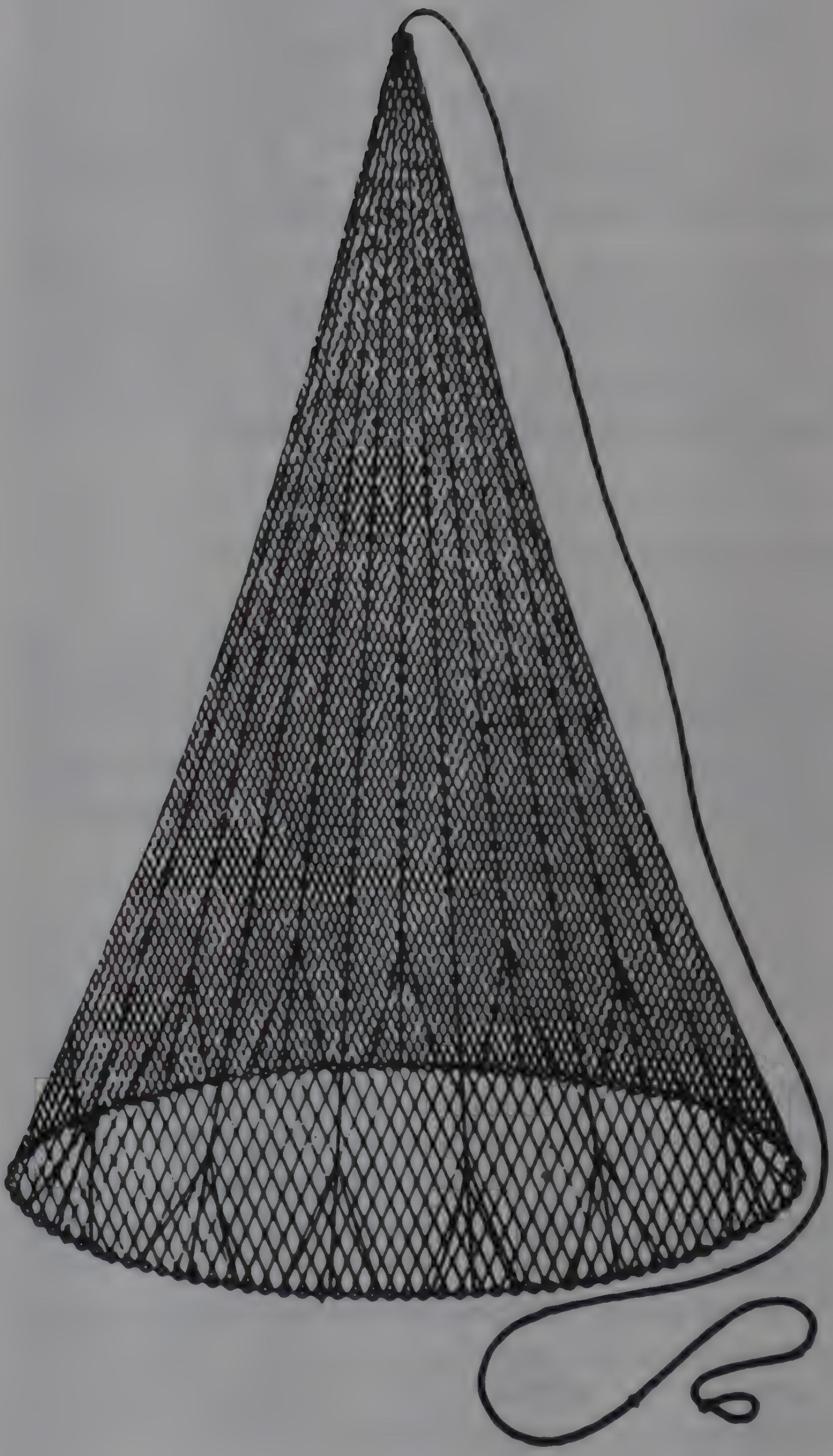

Fig. 3. Stringed cast net.

Mesh Selectivity and Biological Impact Studies on a New Fish-Cum Shrimp Trawl in Palk Bay, Sri Lanka

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SIDDEEK, M.S.M. 1986. Mesh selectivity and biological impact studies on a new fish-cum-shrimp trawl in Palk Bay, Sri Lanka, p. 417-420. *In* J.L. Maclean, L.B. Dizon and L.V. Hosillos (eds.) The First Asian Fisheries Forum. Asian Fisheries Society, Manila, Philippines.

Abstract

Shrimp trawling in Palk Bay, Sri Lanka, is conducted in a 9-m mechanized craft with 30-hp inboard engine. *Penaeus semisulcatus* is the major species. An experiment consisting of twenty trawl operations employing the covered codend technique was carried out using 25, 30, and 40-mm codends in the fish-cum-shrimp trawl nets for mesh selectivity studies. The codend with the 25-mm mesh size hardly released any, whereas others released a fair number of commercially valuable *P. semisulcatus*. The 50% retention lengths for *P. semisulcatus* were 1.96 cm and 2.27 cm for the 30-mm and the 40-mm codend, respectively. When compared with the traditional trawls, the new fish-cum-shrimp trawl with larger mesh size performed better by bringing a slightly larger amount of shrimp and a greater quantity of fish. The instantaneous fishing mortality values during January to June 1984 ranged from 0.16 to 0.38 for *P. semisulcatus* and 0.09 to 0.37 for the bycatch. Yield-per-recruit analysis indicated no immediate threat from the new trawl to the shrimp resources in Palk Bay, but rather a beneficial effect because of the introduction of larger mesh size in the codend.

Introduction

Fishing for finfish and shellfish in the Palk Bay region commenced centuries ago. This region has a shallow flat bottom, averaging 9-m deep, and has been identified as suitable for finfish and shrimp trawling (Malpas 1926; Berg 1971). Among the commercially important shrimp, *Penaeus semisulcatus* is the dominant species found in this region. The finfish catches obtained from Palk Bay are mainly small fish of low commercial value dominated by pony fish.

An experimental trawling exercise was undertaken in April and July 1984 to find out whether the introduction of a new fish-cum-shrimp trawl by the Bay of Bengal Programme has benefitted Pesalai fishermen and contributed to the increase in finfish and shellfish catches in this region. Moreover, it was intended to find out the best mesh size for the codend and the biological impact of this new trawl on the finfish and shellfish populations.

Three types of gear are employed in the shrimp fishery in Palk Bay. Trawling is conducted in a 9-cm mechanized craft with a 30-hp inboard engine, popularly known as a 3.5-t boat. The gill net and trammel net fisheries are carried out in 5 to 7-m fiberglass boats with 8- to 15-hp outboard engines and nonmechanized log rafts. During the study period, there were 66 active craft based at Pesalai and 88 migrant crafts (from Jaffna) based at Talai Mannar. Each year, the migrant crafts remain at Talai Mannar from May to September, which is the peak season for shrimp. The main shrimp grounds exploited by the Pesalai fishermen are in the northeast (ground A) and the southwest (ground B) of Talai Mannar. During May to September fishing activities are intense on ground A, while a fair amount of fishing takes place on ground B from October to March. The trawl fishing for shrimp is carried out mostly during the night, between 6 p.m. and 6 a.m. During certain months when shrimp catches are low, daytime trawling is carried out for pony fish.

Materials and Methods

Three commercial shrimp trawlers of the same class from Pesalai were employed for the mesh selectivity, fishery and biological impact studies. The mean trawling speed of the vessels employed in the experiment was estimated at 2.93 km/hour, while that for the traditional trawl was 2.82 km/hour.

The experiment used three fish-cum-shrimp trawls of the same dimension and construction but with nets of mesh sizes 25, 30 and 40 mm, respectively at the codends. The traditional trawl nets were slightly smaller in size and had different configurations of mesh sizes. The codend mesh size varied from 22 to 25 mm. The codend protective net or 'chafer' was removed before connecting the fine small mesh (10 mm) cover to the codend. There were two iron rings attached to the cover, with a distance between them, to keep ample space between the codend and the cover.

The selectivity experiment was carried out on 24-26 April and 10-13 July 1984. There were seven fishing trips with twenty hauls. The duration of each tow was 3 to 4 hours. Trawling on fishing ground A was carried out at 9- to 12-m depths throughout the experimental period where most of the traditional trawls were engaged in shrimp trawl fishing during this period. The catches in the cover and the codend were weighed and their lengths measured separately by species at the end of each haul. The traditional trawl catches were also sampled to collect the same information. The stretched mesh sizes of the codend and the cover of the experimental trawl were measured at the end of the day's trawling operation and were found to be uniform.

Results and Discussion

The 25-mm codend retained almost all *P. semisulcatus*, while the degree of escapement of *P. semisulcatus* increased as the codend mesh size increased beyond 25 mm. This was also true in the case of the bycatch. For instance, the escapement rites of Leoignathidae, *Sillago sihama*, Gerridae, *Upeneus vittatus* and Theraponidae increased as the codend mesh sizes increased (Table 1).

Fig. 1 shows the selectivity curves drawn by eye for the 30-mm codend and the 40-mm codend, respectively. The 50% retention lengths and the selection ranges (25 and 75% retention lengths) were evaluated following Pope et al. (1975). The selection ranges of both codends were large and had considerable overlap (Table 2), indicating no clear-cut selection process taking place from those two mesh sizes as reported by Gulland (1983). Van Zalinge et al. (unpublished data) gave a selection range of 1.74-1.96 cm carapace length, from a selection curve obtained by the alternate haul technique with a 43-mm codend net for *P. semisulcatus* in the Gulf between Iran and the Arabian peninsula. El Musa (1982) obtained a selection range of 1.04-1.34 cm carapace length, for the Gulf *Parapenaeopsis stylifera* with a 35.2-mm mesh codend net using the covered codend technique. He speculated that this selection range is largely valid for *P. semisulcatus*. However, the present experiment gave higher selectivity length values for the two comparable codend mesh sizes.

Figs. 2a and 2b compare the monthly catch per unit effort values of shrimp and bycatch between the traditional and the new trawls of the 25-mm and 30-mm codend categories. The 40-mm codend catch rates were not considered because of a lack of data for this codend. The increase in catch per unit effort in the fish-cum-shrimp trawls was not observed to be as large for *P. semisulcatus* as one would expect. On the other hand, the catch rates of the bycatch in the fish-cum-shrimp trawls of all three mesh sizes were substantially higher.

The increases in the net earnings by the new trawls were marginal, probably with the exception of the 25-mm codend, when compared with the traditional trawls (Fig. 2c). The reason is that both types of trawls (except the 25-mm codend) catch comparable quantities of shrimp, thus providing the major share of the earnings. The higher earnings obtained by the fish-cum-shrimp trawls may have been mainly due to the higher catches of fish by these trawls.

These results suggest that in terms of economic returns, the new high opening trawl performs better during the lean shrimp season by catching more fish and performs equally well or even a little better than the traditional trawl during the peak shrimp season. The species composition of the bycatch and the length distribution of the fish and *P. semisulcatus* did no show any substantial variation between the new and traditional trawl catches.

The average stocks of *P. semisulcatus* and the bycatch on fishing ground A were estimated by the swept area method. The swept area method requires data on the catch per unit effort, the area swept in a unit effort, the gear efficiency coefficient and the area inhabited by the stock to compute the size of the stock.

The catch and effort data from January to June 1984 were considered for the monthly catch-per-unit-effort estimation. The area swept in a unit effort was calculated from the speed of tow and the width of the horizontal opening of the trawl net while on tow. A value of 0.5 was assumed for the gear efficiency coefficient. The area of ground A was estimated at approximately 80,000 ha. The biomass estimates were made for each month from January to June 1984. Table 3 gives the estimates of the monthly catch per unit effort, density (kg/ha) and the average biomass for *P. semisulcatus* and the bycatch.

Since the estimates of the average biomass concerned the stocks on ground A, it was possible to estimate the monthly fishing mortalities for the partial biomass on ground A. the seasonal fishing mortalities were estimated by the formula C = F x B where B is the monthly average biomass, C is the monthly catch and F is the monthly instantaneous fishing mortality coefficient. The estimated monthly fishing mortality values are also given in Table 3.

Though the 25-mm codend fish-cum-shrimp trawl performed better on catch rates, the introduction of this mesh size may have a long-term injurious effect on the valuable shrimp resource. This is because it retained almost all *P. semisulcatus*, thus, not allowing many of the juveniles and subadults to grow to maturity and spawn. Hence, the 30-mm and 40-mm stretched mesh codend selections were considered in constructing the yield-per-recruit (Y/R) curves. The Y/R curves, based on weights as well as values, were constructed separately for each selection. The Thompson and Bell technique (Ricker 1975) was employed in estimating the Y/R values.

As a first approximation, the average growth rate of *P. semisulcatus* was estimated from the modal progression of the April and July length-frequency data of the 25-mm net where both sexes were pooled. The modal length was found to have increased from 2.25 cm (carapace length) at the end of April to 2.75 cm during the early part of July, giving an average value of 0.25 cm as the monthly growth. This result was assumed to be valid for the exploitable length range of 1.5 to 4.5 cm. The average weight at each length group was also calculated by estimating the mean weight from a sample of shrimp (both sexes pooled) in each length group. The price per grade was also related to the size range in order to estimate the price for each length group.

In order to construct the Y/R curve, a value of 3 for M, accepted as a reasonable value in the Gulf region, was assumed (Van Zallinge et al., unpublished data; Anon. 1982). The average monthly fishing mortality vector on this ground was assumed to be 0.16, 0.16, 0.16, 0.16, 0.38, 0.38, 0.38, 0.38, 0.38, 0.16, 0.16 and 0.16 from January to December. This assumption was made by extrapolating the estimates of F obtained for January to June 1984 to other months, considering the intensity of fishing during those months. Different levels of fishing mortalities were assumed in estimating the Y/R values conforming to the above seasonal pattern.

In the case of the 30-mm stretched mesh codend selection, the Y/R computation was performed assuming 1,000 recruits of 1.25 to 1.50-cm carapace length range entering the fishing ground during March (spring recruits) and dying of natural causes until they attained the size range of 1.75- to 2.00-cm carapace length. Thereafter, fishing mortality acted along with natural mortality according to the mortality pattern given above. In the case of the 40-mm stretched mesh codend, the fishing mortality commenced when the recruits reached the length range 2.25 to 2.50 cm.

Fig. 3a refers to Y/R in weight, while Fig. 3b refers to Y/R in value. In both cases, the Y/R values for the 30-cm stretched mesh codend were higher than those for the 40-mm stretched mesh codend. The present fishing mortality vector 0.16 [4(1), 5(2.38), 3(1)] gives Y/R values at the rising limb of the curve, indicating that there is room for a slight increase in fishing effort without fear of growth overfishing with the new larger mesh codend trawls. It is also important to note that the Y/R curve in weight has no maximum as generally observed in shrimp populations (Anon. 1982). On the other hnad, the Y/R curve based on value for the 30-mm stretched mesh codend shows a maximum at the fishing mortality vector 0.35 [4(1), 5(2.38), 3(1)]. Subject to further investigations of this type, it was tentatively recommended to use a 30-mm codend in the fish-cum-shrimp trawl nets.

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Table 1. Percentage contribution of each species of shrimp and fish in the codend and the cover catches for three mesh sizes of fish-cum-shrimp trawl nets.

Species	25 mm		30 mm		40 mm	
	Codend	Cover	Codend	Cover	Codend	Cover
P. semisulcatus	99.35	0.65	79.58	20.42	68.68	31.32
P. latisulcatus	100.00	0.00	0.00	100.00	29.41	70.59
P. monodon	100.00	0.00	100.00	0.00	–	–
Small prawn	71.57	28.43	4.38	95.62	48.51	51.49
Leiognathidae	94.95	5.05	70.78	29.22	27.58	72.42
Sillago sihama	89.74	10.26	7.95	92.05	54.31	45.69
Gerridae	95.63	4.37	81.66	18.34	60.54	39.46
Upeneus vittatus	100.00	0.00	92.19	7.81	63.87	36.13
Theraponidae	53.65	46.35	40.61	59.39	31.34	68.66
Clupeidae	96.34	3.66	7.05	92.95	48.03	51.97
Seleroides leptolepis	77.70	22.30	100.00	0.00	100.00	0.00
Siganidae	–	–	71.39	28.61	100.00	0.00
Sciaenidae	100.00	0.00	70.48	29.51	–	–
Lethrinidae	100.00	0.00	–	–	100.00	0.00
Anchoviella spp.	4.47	95.53	0.00	100.00	16.67	83.33
Pleuronectiformes	100.00	0.00	100.00	0.00	42.92	57.08
Trygonidae	100.00	0.00	100.00	0.00	100.00	0.00
Loligo spp.	–	–	100.00	0.00	100.00	0.00
Sepia spp.	100.00	0.00	100.00	0.00	100.00	0.00
Octopus spp.	0.45	99.55	26.73	73.27	37.41	62.59
Portunus pelagicus	100.00	0.00	100.00	0.00	98.25	1.75
Large shrimp (total)	99.35	0.65	78.37	21.63	68.21	31.79
Marketable fish (total)	100.00	0.00	100.00	0.00	96.04	3.96
Trash fish (total)	76.02	23.98	49.40	50.60	39.11	60.89

Table 2. Retention and selection data of the new trawl for *P. semisulcatus*.

Codend mesh size	50% retention length (carapace)	Selection range (carapace)	Method
a) 30 mm	1.95 cm	1.50 – 2.35 cm	Free hand drawing
40 mm	2.25 cm	1.70 – 2.75 cm	Free hand drawing
b) 30 mm	1.96 cm	–	Moving average
40 mm	2.27 cm	–	Moving average

Table 3. Seasonal variations of fishing mortality, biomass of *P. semisulcatus* and bycatch in the fishing ground between Pesalai and Talai Mannar (ground A).

	P. semisulcatus				Bycatch			
1984	CPUE (kg/ha)	Density (kg/ha)	F	Biomass (t)	CPUE (kg/ha)	Density (kg/ha)	F	Biomass (t)
J	1.61	1.10	0.16	88.00	4.25	2.91	0.16	232.80
F	1.12	0.77	0.16	61.60	9.46	6.48	0.16	518.40
M	1.05	0.51	0.20	40.80	10.90	5.34	0.10	427.20
A	1.47	0.72	0.16	57.60	14.88	7.28	0.09	582.40
M	1.50	0.73	0.38	58.40	11.08	5.42	0.21	433.60
J	1.92	0.94	0.37	75.20	11.41	5.59	0.37	447.20

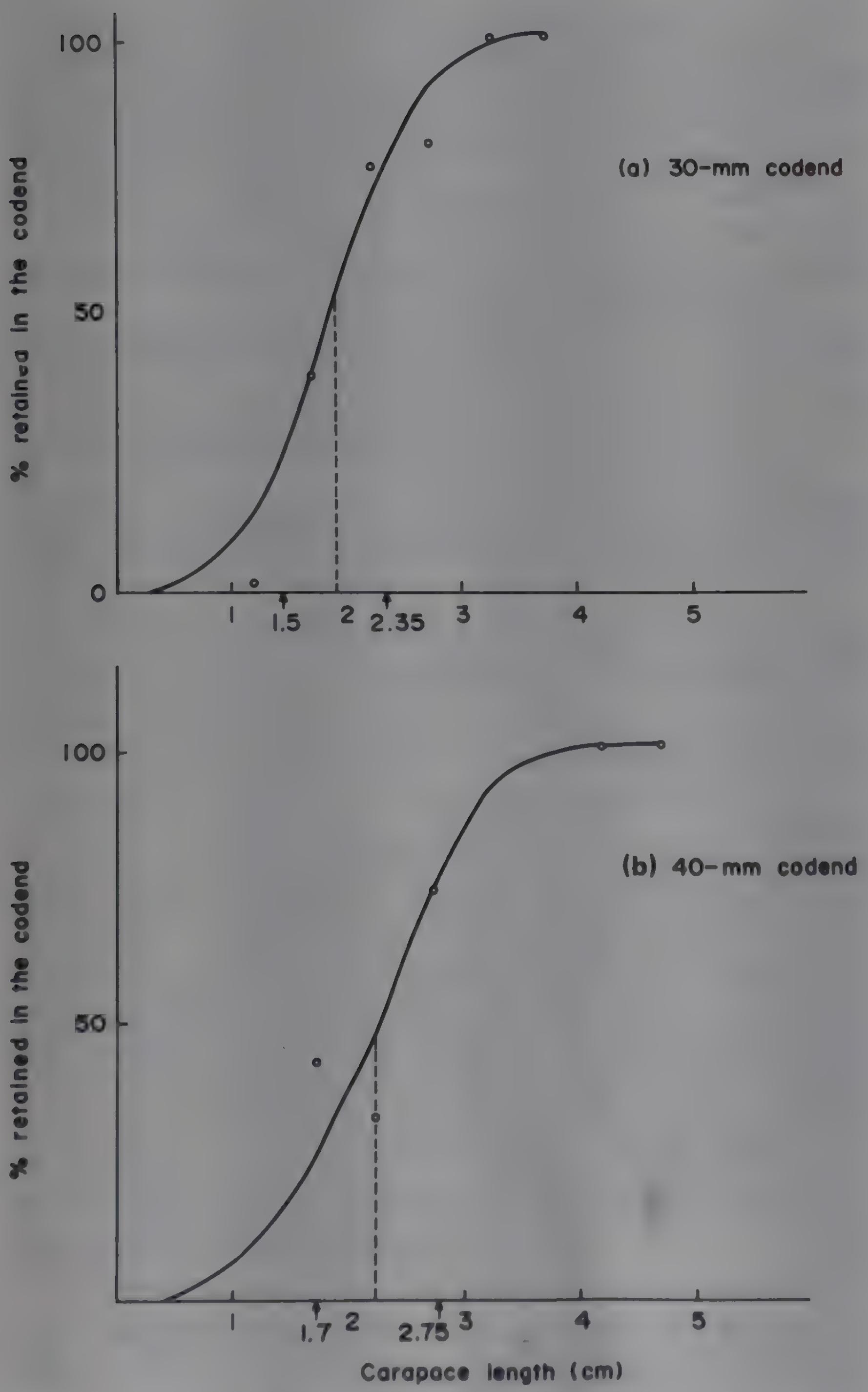

Fig. 1. Selection ogives of *P. semisulcatus* for 30-mm stretched mesh codend (a) and 40-mm stretched mesh codend (b).

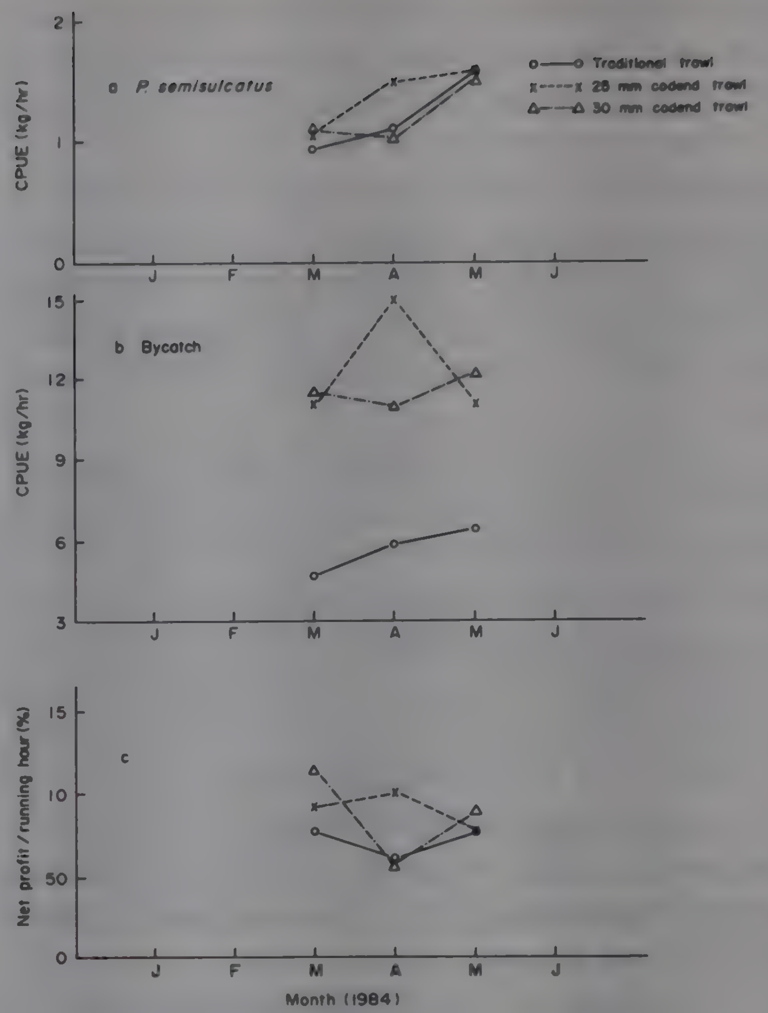

Fig. 2. Comparison of seasonal CPUE values of *P. semisulcatus* (a), by-catch (b), from 25 mm and 30 mm codend mesh of the fish-cum shrimp trawl with the traditional trawl and comparison of seasonal net profit per running hour between them (c).

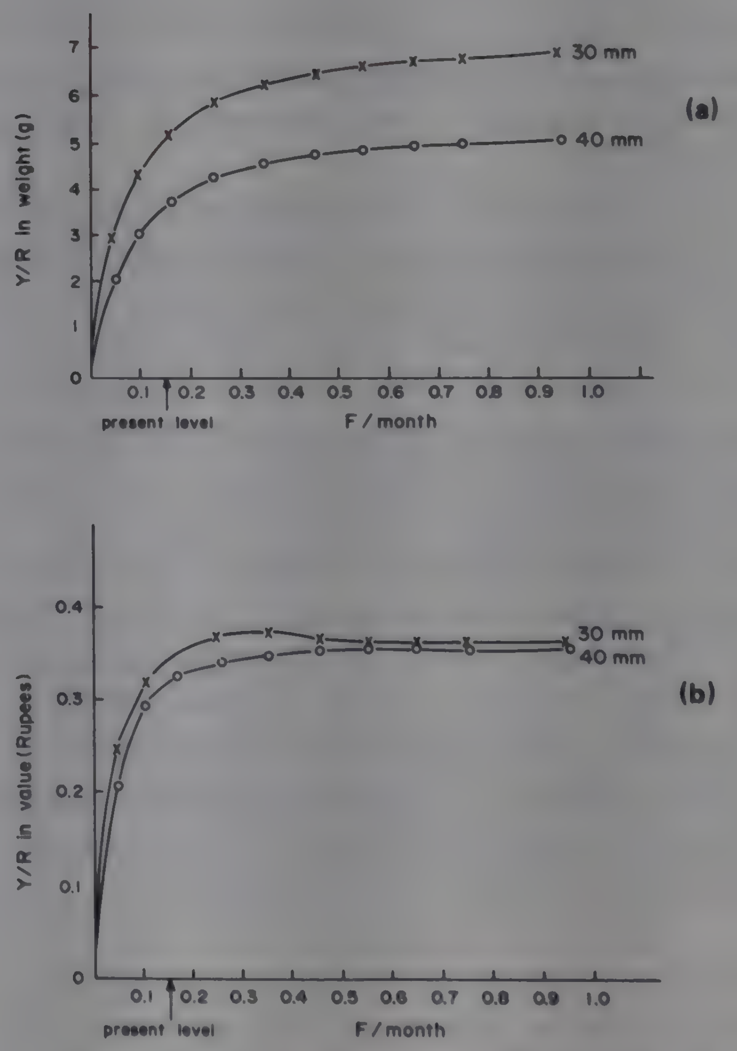

Fig. 3. Variation of yield per recruit of *P. semisulcatus* in weight (a), and in value (b), with F (M = 3/yr).

The Catching Mechanism of a Prawn Pot in Lake Biwa, Japan

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Abstract

The catching mechanism of a fish pot is complex. In this report, attention is focused on the catching mechanism of small prawn pot widely used in Lake Biwa, Japan. To obtain the fundamental catching mechanism, considering the behavior of the target species, both theoretical and experimental studies were carried out. The experimental method employed here was to record the ingress and escape of the prawn in a pot, varying the number of individuals per pot and its construction by changing the funnel shape. To assess the possibility of applying the laboratory results obtained in the lake prawn fishery, the ingress and escape of prawns were also observed in fishing conditions. Good coincidence of calculated and experimental values to evaluate the catching mechanism of small prawn pot is presented.

Introduction

The catching mechanism of a pot is very complex. Research on the pot fishery was made on the relation between pot immersion time and catch (Shinoda and Kobayashi 1969; Bennett 1974), the relation between pot construction and catch (Thomas 1954; Koike and Ogura 1977; Koike and Ishidoya 1978; Koike et al. 1979a, 1979b, 1981; Shelton and Hall 1981) and the relation between ingress, escape, catch and soak (Munro 1974). To date there has been little research on the catching mechanism of a pot (Yoshihara 1957; Inoue et al. 1977, 1978; Hirayama 1981; Yamane and Iitaka 1985). These studies mainly discussed the ingress of the creature into the pot. The pot is so made that the creature can go in and out freely. It is equally important to evaluate the escape behavior of the creature in this study. As a first step, through the variation of environmental conditions, mainly the change in population density in a given space, this study discusses outdoor observations and considers the logic and results of the laboratory experiments.

Methods

Escape is a problem that cannot be ignored in considering the catching mechanism. Escape is a problem related to the variation in the environmental conditions in a pot. Therefore, it is more comfortable to use the phrase "moving ratio" instead of "escape ratio" of the individual.

The moving ratio of creature from a given space A (interior of a pot) to another space B (exterior of a pot) can be defined as:

$$\frac{f(t+1) - f(t)}{f(t)} = -a \qquad \ldots 1)$$

This is known as a first order difference equation. If equation (1) is solved under the condition t = 0, N = N_0, equation (2) is obtained as the solution:

$$f(t) = (1 - a) = N_o \qquad \ldots 2)$$

Here, f(t) is the number in a pot and t is the elapsed time after asymptotic condition in a pot, N_0 is the catch (numbers) at t = 0 and N is asymptotic catch where -a is the moving ratio. If there is no predation between the individuals captured in a pot, the variations in the number of creatures in a given space (interior of a pot) can be calculated through equation (2).

In laboratory experiments, six models were constructed out of plastic basket with the body of an actual pot (Fig. 1). Six entrance funnels with 1.5-cm diameter neck were made using polyvinyl chloride 0.3-mm thick. Height of neck above base had six variations (Fig. 1). The experiments were performed without bait in a still-water tank 1 m x 40 cm high x 30 cm deep. The striped prawns *(Palaemon paucidens)*, 25-50 mm in body length caught by commercial pots and bamboo screen were used as test animals. The numbers of prawns per pot were 50, 100, 150, 200 and 250. Activities near the entrance were recorded with a video camera for 3 hours per experiment. The water temperature was maintained at 20 ± 2°C and light condition was controlled at 300 lux on the water surface.

A series of experiments was performed with six model pots in the fishing ground (2-3 m water depth) in Lake Biwa. Height of neck above base varied in six heights (Fig. 1). The pots used were the same as those used in the laboratory. The number of prawns per pot varied from 20 to 150 according to the number of prawns available (22-45 mm in body length). Pots were set without bait at the bottom at intervals of about 2.5 m. The number of prawns per pot was counted every 2 hours (9 a.m. to 5 p.m.) for each pot when hauled. A flap was attached to the base of the pot to prevent prawns from escaping. This flap was designed so that the opening was closed during hauling and setting of the pot. Water temperature during experiment was 18-29°C.

Results and Discussion

In laboratory experiments, as first step to test the validity of the method proposed in this study, the experimental values and the calculated values of the moving ratios were compared (Fig. 2). The model equation satisfactorily fitted the changes over time in the number of prawns remaining in the pot after the beginning of the experiment in almost all cases. The mean moving ratios in Table 1 for 10 min. duration were used in the calculation. It seems obvious that the variations in the number of individuals in a pot could be represented with the use of equation (2).

A series of field experiments tested the applicability of the evaluation method used under laboratory conditions and the results compared with theoretical values (Fig. 3). The variations in the number of creatures in a pot for a duration of 6 hours are well defined by equation (2). The mean moving ratios in Table 2 were used in the calculation.

Next, a series of comparison of both the laboratory and field experimental values based on the moving ratios and height of neck above base, was done. The calculations were based on data from 100 and 150 individuals only as the results on other population densities in the field experiments under different conditions could not be obtained owing to unavailability of test animals (Fig 4). The moving ratios from the laboratory tests became large compared with the results obtained from the field tests. Although the plots in the graph are somewhat scattered, the values of field and laboratory experiments show almost similar trends. For the present study, the effect of the height of neck above the base to the escape behavior of individuals is minimal.

The spatial behavior of the individuals in a given space may be one of the important factors in considering the catching mechanism. Research workers (Morishita 1954, 1971; Shigesada and Teramoto 1978; Shigesada 1980) have pointed out that there is a population pressure among individuals which accelerates dispersion. In equation (2), the value of -a represents a probability of movement of individuals from given space A (interior of a pot) to another space B (exterior of a pot). In this study, the relation between the value of -a and population pressure is not clear.

The good coincidence of calculated and experimental values to evaluate the catching mechanism of small prawn pot shows the validity of the method proposed in this study.

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Table 1. Mean moving ratios of prawns in relation to the pots during laboratory tests.

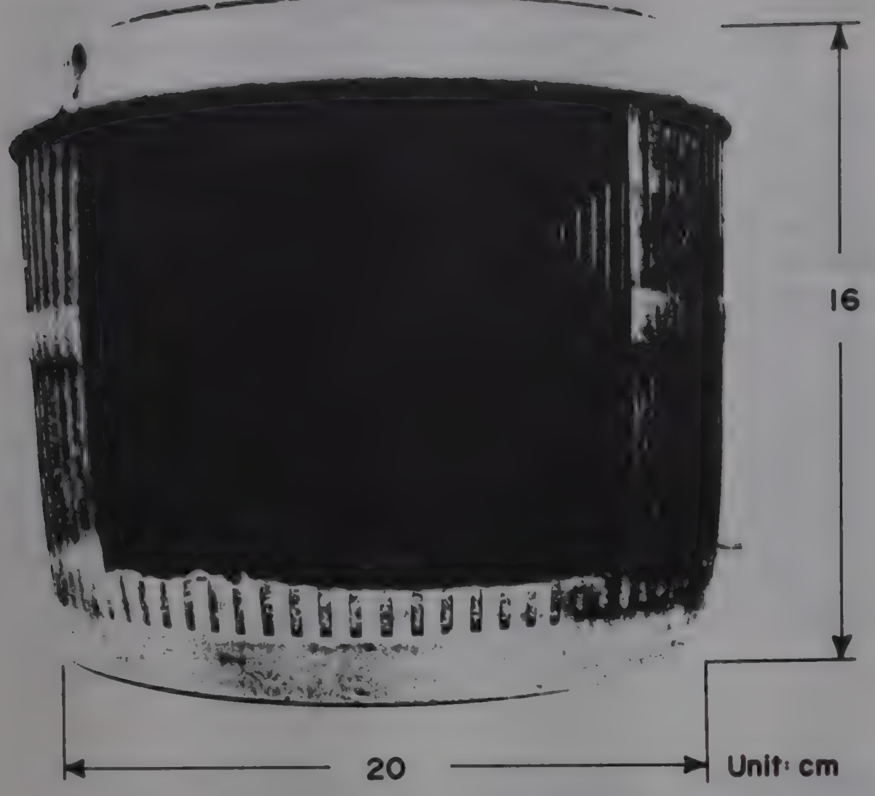
	Height of neck above the base (cm)					
No. Prawns	2	4	8	10	13	16
50	*	0.0056	0.012	0.022	*	0.044
100	0.0056	*	0.0071	0.0069	0.0067	0.0067
150	0.011	0.022	0.0035	0.0052	0.0031	0.0022
200	*	0.0048	0.0052	0.0090	0.0022	0.0054
250	0.0056	0.0021	0.0067	*	0.0029	0.0042

*Moving ratio is zero.

Table 2. Mean moving ratios of shrimp in relation to the pots during field tests.

	Height of neck above the base (cm)					
No. Shrimp	4	6	8	10	12	14
20	0.0059	0.0035	0.0083	0.0083	0.0045	0.0011
40	0.0041	0.0023	0.0035	0.0041	0.0083	0.0011
60	*	0.0083	0.0067	0.0019	0.0015	0.0027
80	0.0096	0.0080	0.0029	0.0059	0.0050	*
100	0.0033	0.00095	0.0033	0.0035	0.0019	0.0016
150	0.0038	0.0036	0.0052	0.00063	0.0038	0.0020

*Moving ratio is zero.



Height of a neck above the base (cm)

Laboratory test	2	4	8	10	13	16
Field test	4	6	8	10	12	14

Fig. 1. Experimental prawn pot.

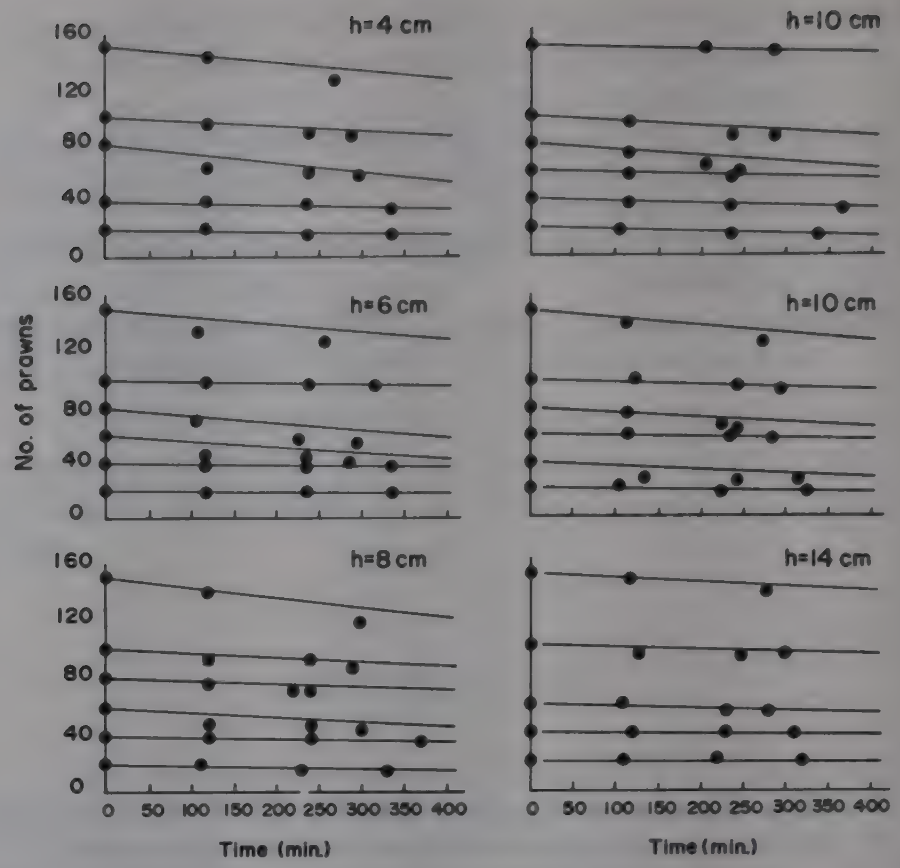


Fig. 3. Variation in the number of individuals in a pot at different prawn densities for field experiments. h = the height of neck above the base.

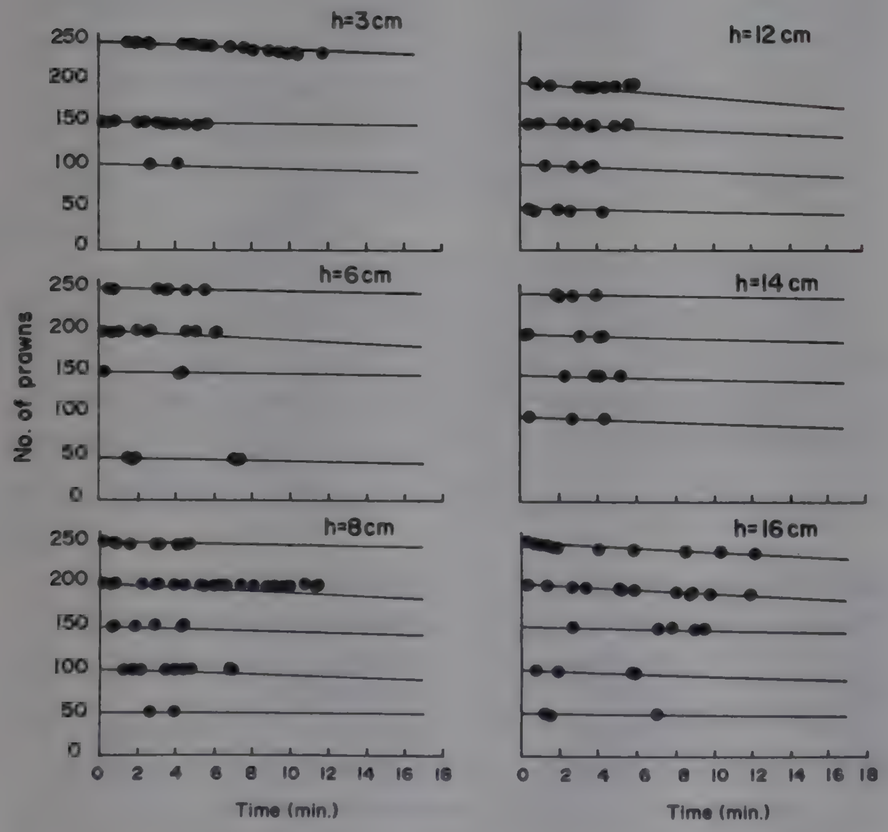


Fig. 2. Variation in the number of individuals in a pot at different prawn densities for laboratory experiments. h = the height of neck above the base.

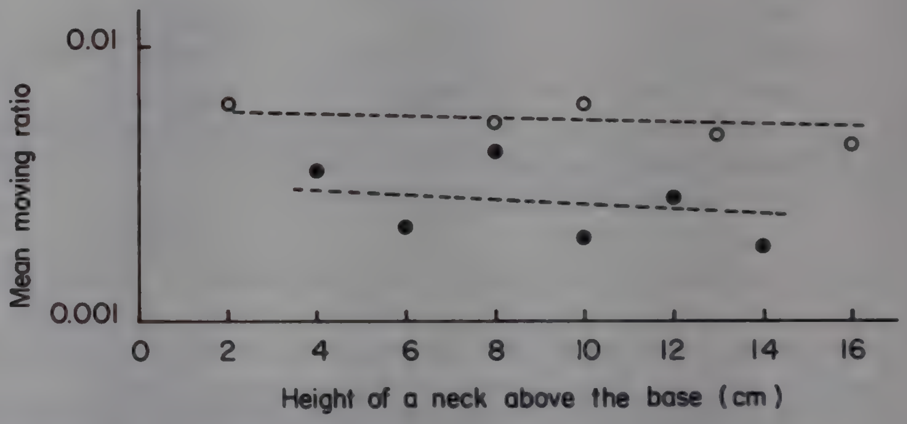


Fig. 4. Comparison of mean moving ratios. Closed circles represent the results for field tests and open circles represent the results for laboratory tests.

Computerized Literature Searches: Their Advantages and Limitations

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Abstract

To obtain some measure of the effectiveness of computerized literature searches, relative coverage, overlap and cost were quantified for the ASFA and BIOSIS databases. Of the 7,800 baseline references in a bibliography on the carp *(Cyprinus carpio* L.*)*, less than half were computer accessible. Although in recent years up to 85% of known publications were retrieved by the combined databases, the low overlap (14%-26%) between these two information systems meant that retrieval rates for either database rarely exceeded 50% of the pertinent articles published annually. The BIOSIS computer search required 15 minutes to perform, but 100 hours to replicate manually; thus manual citation retrieval would have been economically justified at labor costs of less than US$7/hour. Citation errors, a considerable time-lag prior to database entry and little access to "grey" literature also reduced the value of these literature searches.

Introduction

Computerized database searches are now a key tool for fisheries scientists, providing instant access to the world's fisheries research and management information. Comprehensive literature searches have become imperative to avoid duplication of research. However, such searches are costly and may be difficult to arrange throughout Asia. Likewise, many workers regularly assume that such searches extract all the significant references on a particular subject. This may be far from true. Therefore, in order to provide a practical measure of the cost-effectiveness and efficiency of computerized searches, several major databases were quantitatively evaluated for coverage, overlap and cost.

Methods

A "complete" bibliography of the scientific literature on the common carp *(Cyprinus carpio* L.*)* was compiled as a baseline for coverage evaluation. The two largest fisheries-related databases, BIOSIS (BioSciences Information Services, Philadelphia, Pennsylvania USA, 1969+) and ASFA (Aquatic Sciences and Fisheries Abstracts, Cambridge Scientific Abstracts, Bethesda, Maryland USA, 1972+) were searched via computer through the DIALOG network (Lockheed Corporation, USA) employing the keywords "Cyprinus" and "carp." The hard copies of these databases (Biological Abstracts, 1927+; Current Bibliography for Aquatic Sciences and Fisheries, 1958-1971 (FAO, Rome), Aquatic Sciences and Fisheries Abstracts, 1972+, respectively) were also searched manually through the printed indexes using traditional, noncomputerized methods and the same keywords. A similar manual search was performed on the Asian regional bibliographic journal, AGRIASIA (Agricultural Information Bank for Asia, SEARCA, College, Laguna, Philippines, 1977-1985).

Additional references were obtained from citations listed in books and scientific articles which had been acquired following identification by computer or manual searches.

Spurious references and citations referring solely to other carps (e.g., grass carp, *Ctenopharyngodon idella* Val.*)* were removed. For example, BIOSIS identified articles in the *Ice Cream Makers' Journal* (Miscitation of: Das and Subla 1964), *Entomologische Blaetter fuer Biologie und Systematik der Kaefer* (Dahlgren 1981) and *Contraceptive Delivery Systems* (Zhang and Liu 1982) which proved nonexistent or irrelevant to the carp.

To test the completeness of the resulting bibliography, references cited in the then unexamined Hatchery Manual for the Common Carp (Jhingran and Pullin 1985) were checked for inclusion. No new scientific articles on the carp were found. Therefore, the compilation appeared to be reasonably complete for more recent scientific papers. No adequate benchmark could be identified to evalute the completeness of earlier references (i.e., pre-1945).

The determination of "acceptable" articles was partially subjective in nature (i.e., should a passing reference to carp be sufficient justification for entry?) and a few additional unaccessed references are still being identified, therefore, data should not be considered final, but as representative of the situation in January 1986.

Results

Slightly over 7,800 articles on the common carp were identified from over 1,200 journals. BIOSIS provided computer-based access to 2,900 of these articles; ASFA accessed 2,100 (Table 1). Manual retrieval techniques produced 84 articles from AGRIASIA. From 1972 to 1984, BIOSIS and ASFA provided computerized access to between 71% and 85% of pertinent articles published annually. For the same period, only 20% (range: 14%-26%) of these computer accessed articles were shared by both databases (Fig. 1).

Total cost of both computer searches was US$1,400 (in 1985) for citations alone; printing of available abstracts would have increased the cost to US$2,000.

Manual searches of both BIOSIS and ASFA returned identical references to those retrieved by computer for years when both methodologies existed. On average, 25 citations per hour from either bibliographic journal could be manually retrieved. The BIOSIS computer search required approximately 15 minutes to perform, but about 100 hours to replicate manually.

Manual searching of the bibliographies revealed the presence of considerable numbers of articles pertinent to carp that had not been accessed by the keywords, either by computer or via the index of the bound bibliography. For example, even without comprehensive searching, such unaccessed articles still comprised 5-14% of the articles actually computer accessed by ASFA each year (Table 1).

Spelling or copy errors were present in slightly less than 1% of the citations regardless of source or format. Multiple citation of the same article also occurred regularly (e.g., ASFA Part 1, 1978 citations #1729 and #9313 both cite Nakamura and Kasahara 1977). Mis-citation of page numbering occurred in approximately 10% of the BIOSIS references.

Neither bibliography, either in electronic or hard-bound format, provided access to articles earlier than six months after their publication, with the observed median entry time being a little more than one year.

Unpublished institutional literature, the so-called "grey" literature, was seldom cited by either major database. Neither contained more than 10 items of grey literature pertinent to carp.

Discussion

Obviously, the results obtained here for literature on carp may require re-interpretation for application to more recently developed research areas having less pre-database material.

Significantly, the combined computer searches of ASFA and BIOSIS failed to retrieve even half of the massive literature on this fish, due both to the presence of considerable material published prior to initiation of the databases and incomplete coverage of the modern literature. At first glance, the recent 71-85% coverage completeness appears promising, but at best each individual database accesses only slightly more than half the relevant articles published annually. This interpretation is conservative, since additional unaccessed literature is still being uncovered which is unlikely to be subsequently entered onto these computer systems.

Considerable overlap between the major databases was originally expected. The relatively low level of citations held in common suggests dissimilar specialization by each bibliographic system. ASFA describes its area of expertise as "... an international information journal for the science, technology and management of marine and freshwater environments" while BIOSIS contains "worldwide research in life sciences". These vague generalities suggest that ASFA should be a subset of BIOSIS and that BIOSIS should contain everything published on fish, including carp. ASFA tended to contain more practical and applied fisheries work while BIOSIS was oriented more towards "pure" scientific and theoretical material. One might also view this dichotomy as representing the basic difference between academic research versus task-oriented governmental or commercial research.

Until the overlap between ASFA and BIOSIS fisheries citations is significantly increased, it appears that both databases must be searched to provide a reasonably complete current literature compilation. Unfortunately, the need for dual searches seems especially likely in aquaculture where many fields, such as nutrition and disease, tend to have both theoretical and applied components.

Given the cost of these searches and the manual retrieval rate, it would be cost-effective to perform a search manually if available labor was priced below US$7/hour. Further, manual searching resulted in citations on index cards whereas the computer printout was unordered and required transfer to cards to be of continuing use. Thus, the ceiling cost for manual searching may actually be underestimated.

The presence of many pertinent articles within each database which were not accessed was both surprising and disappointing. It would appear that the current system for retrieving citations by entering keywords and taxa is imperfect. This implies that the problem of incomplete literature access, while apparently due to the breadth and effectiveness of journal coverage, is actually partially due to incomplete internal retrieval of material which is already resident within the system.

The quantity of citation errors noted suggests that authors of scientific articles would be unwise to rely on these bibliographic citations, as opposed to actual copies or reprints of articles, for compiling reference lists for publications. The problem of incorrect citation of page numbers by BIOSIS appears to be a result of obtaining this information from the table of contents of each journal, rather than from actual copies of the articles. ASFA material rarely exhibited this problem.

Given the time-lag between research and publication, and publication and database entry, it appears that "recent" material available on databases actually represents, at best, the forefront of research three years prior. Short of the development of direct electronic database entry by the journals (or researchers) themselves, workers should view retrieved material as a means of identifying key researchers who can be contacted to determine the current state-of-the-art, rather than the last word on recent findings.

With ever-increasing publication costs, it is becoming less common to see articles containing extensive data sets, despite the fact that data, not discussion and interpretation, represent the real findings in science. Routine data such as annual site-specific catch, age structure and species composition are increasingly being relegated to the "grey" literature. It is apparent from this work that these two databases are not accessing much of this material, especially since a worldwide survey conducted on the common carp yielded a considerable amount of grey literature (i.e., a 0.65-m stack). While this may be beyond the scope of operation of these repositories, it still denotes the omission of a major resource which is not being adequately addressed in any other vehicle(s).

These results infer that computer database searches should best be viewed as partial subsamples of the existing literature. The primary advantage of computerized searches is their ability to provide a very rapid reference base as an introduction to a new research area, thus facilitating communication with key researchers. However, computerized database searches are no replacement for continuous and long-term comprehensive literature acquisition.

Acknowledgements

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Table 1. Total number of computer accessible articles on carp published in recent years in ASFA, BIOSIS, or both, as well as pertinent citations present in ASFA which were not accessed. Comparative citation rates for AGRIASIA and overlap with the other two major databases are also presented.

Year	ASFA	BIOSIS	Overlap	Unaccessed (ASFA only)	AGRIASIA	Overlap
1984	285	301	122 (26%)	27	12	0
1983	183	213	75 (23%)	20	10	1
1982	133	204	50 (17%)	10	11	0
1981	176	206	62 (19%)	19	10	1
1980	181	211	71 (22%)	13	13	1
1979	159	208	65 (22%)	14	6	0
1978	149	204	51 (17%)	14	5	0
1977	202	173	56 (17%)	12	13	1
1976	101	189	36 (14%)	7		
1975	145	200	54 (19%)	21		
1974	145	133	54 (24%)	7		
1973	158	150	44 (17%)	17		
1972	116	122	41 (21%)	13		

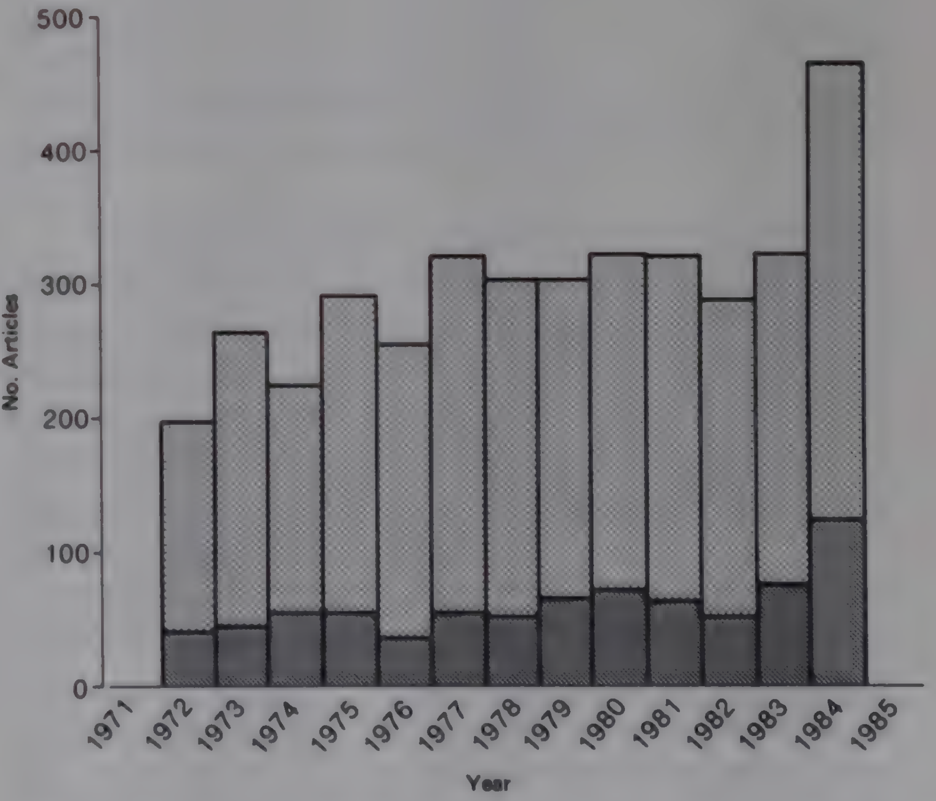

Fig. 1. Total annual computer accessible citations in either ASFA or BIOSIS (light shading) and the numbers held in common (dark shading).

Bacterial Depuration of Grossly-Contaminated Oysters, *Crassostrea iredalei**

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Abstract

Oysters *(Crassostrea iredalei)* from a commercial growing area in Capiz, Iloilo, Philippines, were purchased from the Iloilo City Central Market and used in a depuration trial within 24 hours of collection. Total coliform (TC) and fecal coliform (FC) levels were determined using the five-tube, most probable number (MPN) technique. Samplings were carried out in three areas in the tank: (a) near the water trickle area, (b) at the middle and (c) near the water outflow area. FC proved to be a better and more consistent indicator of depuration efficiency than TC which gave erratic levels in the first 24 hours. The oysters with initial FC MPN of 2.2×10^5/100 g meat depurated to acceptable levels (<230 MPN/100 g meat) after 48 hours except those in the middle of the tank (490 MPN/100 g). This suggests the presence of an "indifferent" or "dead" spot. Nevertheless, the same oysters depurated successfully within 72 hours. Ranges of chemical and physical parameters in the depuration water were: temperature, 27.0-29.5°C; salinity, 30.5-32.0 ppt; and dissolved oxygen, 4.0-6.2 mg/l.

Introduction

Bivalve molluscs such as oysters, mussels and clams accumulate and transmit many diseases to man when ingested raw. These diseases range from mild forms of gastroenteritis to the more serious ailments such as typhoid fever, cholera, caused by bacteria (Earampamoorthy and Koff 1975) and polio and hepatitis A, which are due to viruses (Gerba and Goyal 1978).

Research during the past half-century has devised depuration systems for cleansing these bivalves of their potentially pathogenic microbial loads prior to sale and consumption. Bivalves are held in tanks of clean or sterilized water to induce them to eliminate with the feces the microorganisms previously picked up or to reject other microbial loads with the pseudofeces.

Most studies initially focused on the elimination of the common pollution indicator *Escherichia coli* before considering other bacteria and viruses. The processes of elimination of *E. coli* had been thoroughly worked out, e.g., in a hard clam, the northern quahaug, *Mercenaria mercenaria* (Cabelli and Heffernan 1970a; Heffernan and Cabelli 1970, 1971; Timoney and Abston 1984); in the soft clam, *Mya arenaria* (Cabelli and Heffernan 1970b); in the eastern oyster, *Crassostrea virginica* (Haven et al. 1978); and in the Sydney rock oyster, *C. commercialis* (Fleet 1978; Souness et al. 1979; Eyles and Davey 1984; Rowse and Fleet 1984).

The Philippine slipper oyster, *Crassostrea iredalei*, is known to cause various forms of gastroenteritis and other stomach ailments sporadically. This paper reports the results of a depuration run under normal conditions in Iloilo, Philippines, specifically in the portion of Guimaras Strait fronting the site of the Southeast Asian Fisheries Development Center (SEAFDEC).

Materials and Methods

Two sacks of oysters *C. iredalei* harvested from a culture farm in Pontevedra, Capiz were purchased from Iloilo City public market at 6 a.m. on 4 October 1984 and transported to the SEAFDEC shellfish depuration laboratory in Tigbauan, Iloilo. These were harvested from the growing area (salinity ≅ 24 ppt) at 4 pm of the previous day, put in moist jute sacks unwashed and trucked to Iloilo City.

Upon arrival in the laboratory, the oysters were rinsed of mud and silt thoroughly with freshwater by means of a jet-spray hose. Adhering barnacles and other fouling animals were removed with a sharp scalpel. The cleaned oysters were arranged individually in 15 plastic trays (30 x 36 x 10 cm) at a density of 20 pieces per tray. The trays were loaded on a rectangular depuration tank and arranged in a 3 x 5 array.

The tank (Fig. 1) was made of 20 mm marine plywood coated on the inside with fiberglass resin (volume ≅ 790 liters). Water entered the tank at 7 l/min. and trickled through a perforated PVC pipe placed at the proximal end of the tank (Fig. 2). At the distal end, water is drained through a 2.5-cm overflow pipe located at a height level with the tank floor (Fig. 3). The volume of the water during the depuration run (depth ≅ 24 cm) was approximately 450 l. At this volume, the water volume to oyster number ratio was 1.5:1.

Water entering the tank was prefiltered with a P-5 polypropylene cartridge of the dirt/rust type (Fig. 1) capable of removing particles of 5 μm (Ametek PS-S$_1$, USA). The filter was replaced with a clean and dry one every 24 hours. The water was disinfected further by passing it through a UV sterilizer (Oliphant Ultra-Flo Liquid Sterilizer, Australia).

About 6-8 medium-sized oysters were picked at random and pooled for total coliform (TC) and fecal coliform (FC) levels using the most probable number (MPN) method as advocated by the American Public Health Association (1977). For the 0 hour analysis, only one pooled sample was used. Subsequently, oysters samples were picked from three different areas in the tank (Fig. 4). Sampling was done every 24 hours up to the 96th hour. Pre-filtered and UV-sterilized water as collected from the trickle before hitting the tank were also analyzed every 24 hours.

On the 25th, 49th and 73rd hour or right after sampling, the depuration process was disrupted. The seawater was evacuated; the tank bottom and sides, the plastic trays and the oysters were sprayed thoroughly with freshwater to remove feces and pseudofeces accumulated on the bottom and on the sides. Depuration was then resumed immediately.

Results

The sample oysters were grossly contaminated judging from the initial total coliform (TC) and fecal coliform (FC) most probable number (MPN) of 2.2 x 10^5/100 g meat. It was rainy at the time the oysters were harvested, which partly explains the high initial MPN. The high initial MPN level may have been caused also by unsanitary handling. Between harvest and the initial MPN determination, approximately 18 hours elapsed over which period the bacteriological load may have increased to the observed initial coliform MPN. The FC is known to increase considerably during the interval between harvest and arrival at the wholesale market (Sbaih et al. 1984).

The 24- and 48-hour data (Fig. 5) show that oyster nearest the trickle area in the tank, A, cleansed themselves fastest; those in the middle, B, the slowest (Fig. 4). These suggest the presence of a "dead" or relatively stagnant volume located in the middle.

The MPN data in B on the 48th hour puts the appropriateness of the whole tank in question. Although the oysters on both ends were sufficiently cleansed to a level lower than the acceptable MPN 230/100 g meat, those in the middle were not until 72 hours depuration.

The ranges of chemical and physical parameters in the depuration water used throughout the experiment were as follows: temperature, 27.0-29.5°C; salinity, 30.5-32.0 ppt; and dissolved oxygen, 4.0-6.2 mg/l.

Discussion

Up to the 48th hr, the acceptance level had never been reached when TC was used as the indicator of the depuration process. Future work therefore, should utilize FC levels instead of TC as the indicator.

On the basis of the initial FC level of MPN 2.2 x 10^5/100 g meat, elimination rates were calculated to range from 99.78 to 99.98% after 48 hours, up from 99.27 to 99.91% observed after 24 hours. Findings of Haven et al. (1978) from whom the present testing tank was patterned, generally showed elimination rates of between 93 and 98% in the first 24 hours.

Experience in Great Britain and in the USA on *Crassostrea virginica* showed that a depuration time of 36 to 48 hours is enough to cleanse oysters to acceptable levels (Haven et al. 1978). In Australia, with *C. commercialis*, the depuration process is for 36 hours as legislated (Souness et al. 1979; Rowse and Fleet 1984).

In this depuration run, cleansing to the acceptable FC level was achieved with a very conservative flow rate of 7 l/min. which is less than 1/3 of the rating of 22.5 l/min. or a flow rate of 35 l/bushel (or 300 oysters)/min. for this size of tank (Haven et al. 1978). Only 420 l of the water was used every hour of the 1,350-l volume required when the full rates are fulfilled. In recirculating tank systems in Australia, flow rates are such that at least two complete water changes are effected in an hour (Souness et al. 1979; Rowse and Fleet 1984).

The disruption in the depuration process on the 25th, 49th and 73rd hour, or right after daily collection of oysters for sampling, was instituted to flush away biodeposits, feces and pseudofeces, which sedimented on the tank bottom or attached to the tank sides, or on the plastic trays. Any drastic change in tank conditions such as slightly elevated temperatures eventually leads to the collapse of the binding fecal mucus, loosening apart the encased bacteria which may be rapidly buoyed up and taken again by the oysters. This is generally indicated by an increase in the FC level, which may be higher than the initial level (Gallego et al., unpublished data). This "rebound" had been observed by other workers (Furfari 1966; Haven et al. 1978; Timoney and Abston 1984) with *C. virginica*. It was therefore recommended by some workers that depuration tanks be drained at 24-hour intervals, the oysters taken out and the tanks hosed down with treated seawater to remove such biodeposits (Furfari 1966). Other workers such as Devlin and Eng (1973) and the SYNCD (1969) do not agree to the washdown, however. It is argued that draining and flushing of tanks periodically may resuspend the detritus during the refilling, leading to increases in bacterial levels in both seawater and oyster.

The lag in the cleansing action among oysters located in the middle of the tank in the present experiment indicates a relatively stagnant or dead volume (DV) of water. This is basically an engineering problem, which may be remedied in many ways such as relocation of the trickler to another area, repositioning of water flow directions or alteration of water depth. A water evacuation and replacement study conducted by SEAFDEC engineers showed that the DV of this particular tank at a water volume of 512 l was approximately 80 l or 15.7% without oysters and 128 l or 27% with oysters (A.T. Vizcarra and P. Gavieta, pers. comm.) suggesting a "mixed" type of flow instead of "plug" flow. The depuration lag in a portion of the tank also explains why previous work done by our group on pooled samples showed unacceptable FC levels after 48 or even 72 hours (Gallego et al., unpublished data).

With some minor alterations accorded the tank, the water flow patterns may be improved to assure cleansing of the FC to acceptable levels in 48 hours or less. Although bacteria are the target of depuration, other disease-causing microorganisms such as viruses are also depurated. A number of workers typified by Furfari (1966) and Gerba and Goyal (1978) believe that viruses are generally eliminated in the same manner and duration as the bacteria.

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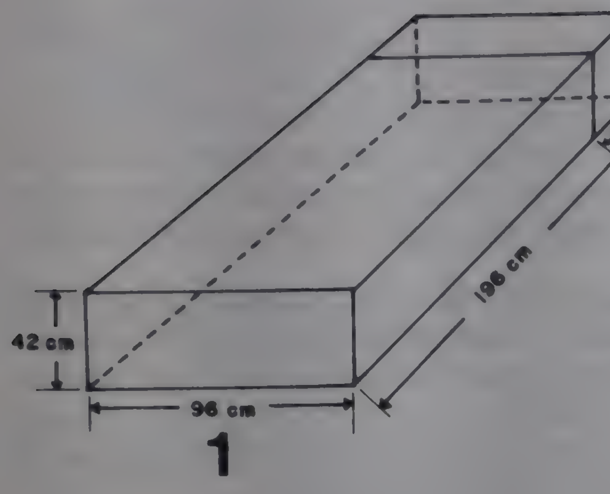
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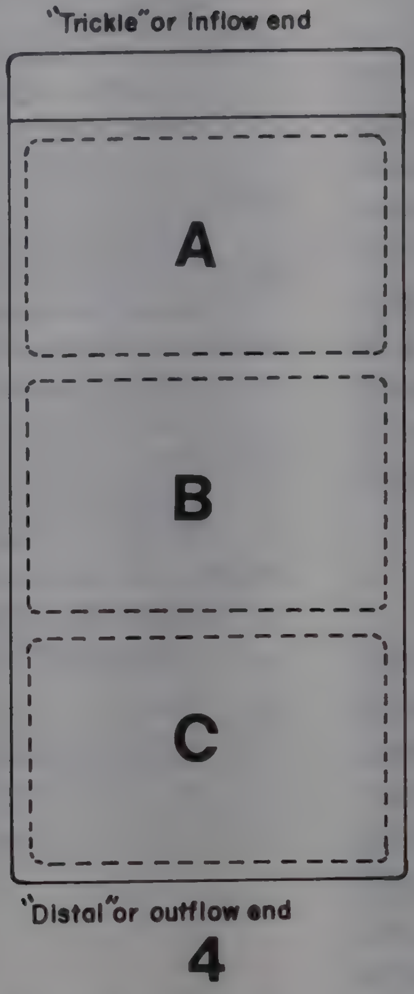
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B
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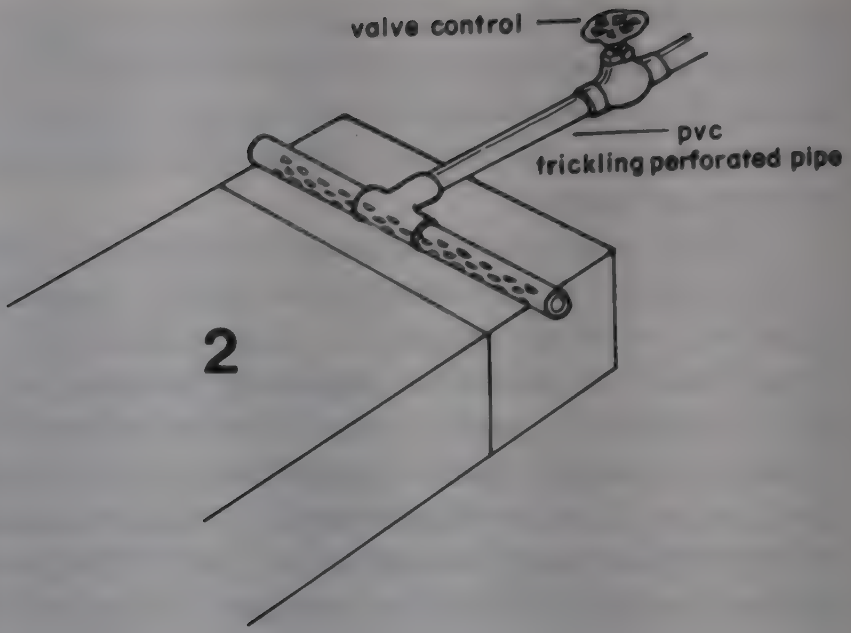
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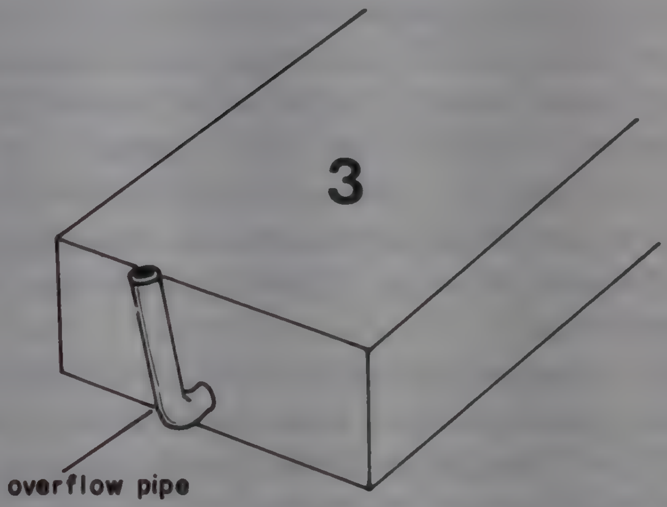
3
overflow pipe

Fig. 1. Depuration tank of marine plywood used in the experiment.

Fig. 2. Water trickles through this perforated pipe.

Fig. 3. Overflow pipe where water is drained.

Fig. 4. Depuration tank areas: A, the inflow area nearest the water trickle; B, the middle area where oysters cleansed the slowest; C, the outflow end.

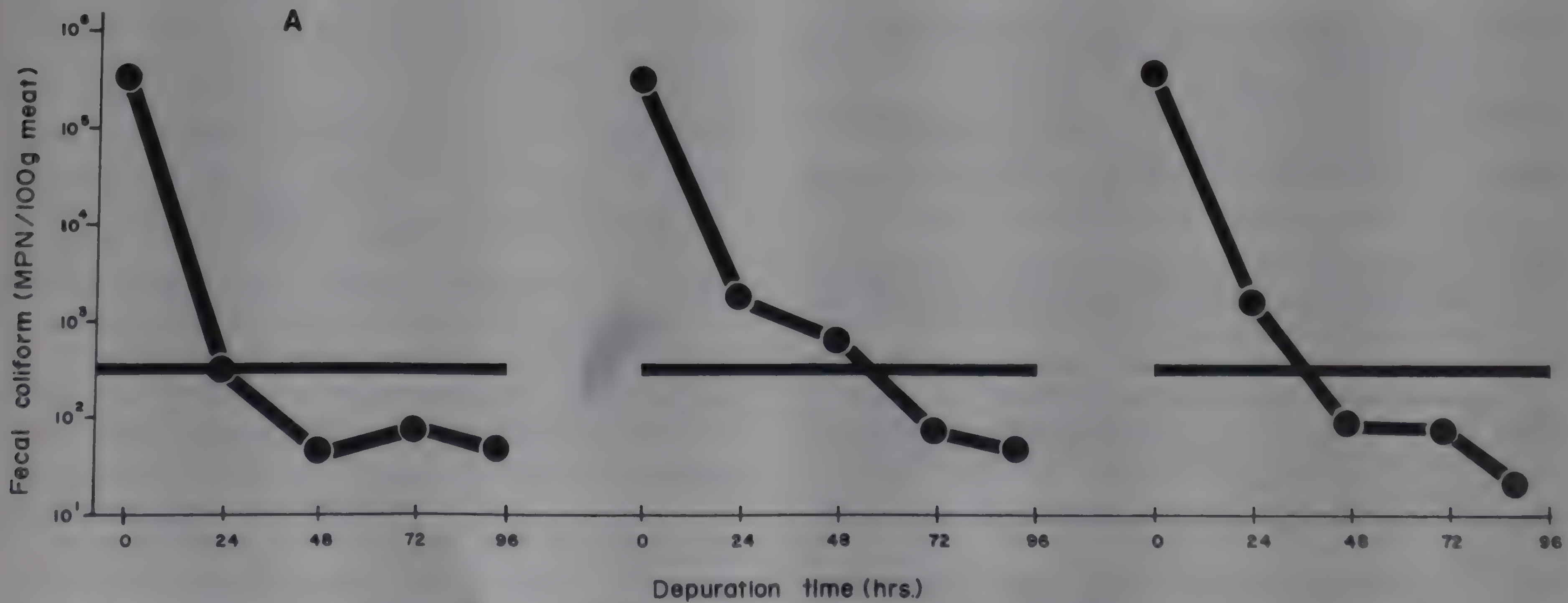

Fig. 5. Changes in the fecal coliform (FC) load (MPN/100 g meat) in the slipper oyster *Crassostrea iredalei* from three areas of a 4 x 8 m wooden tank during depuration over a period of 96 hours. The areas sampled and their respective depuration curves were: A, near the trickle; B, at the center; and C, near the outflow area. The horizontal bar at log 2.36 represents the acceptance level (or 230 MPN/100 g meat).

Tilapia Marketing Tests in Kuwait*

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Abstract

In 1982, live tilapia were introduced into the Kuwait markets for the first time as part of a project to determine their aquaculture potential in Kuwait. Further market tests were held in 1983, 1984 and 1985. Various sizes (250-900 g) and two color patterns (natural coloration and red) of tilapia were sold and consumer response measured. Kuwaitis were initially cautious because the fish were unknown to them. Other consumers, particularly Egyptians who were familiar with tilapia, enthusiastically purchased the fish. In the later years, Kuwaitis' acceptance of the fish increased substantially. Large tilapia were preferred by Kuwaitis while the Egyptians preferred smaller fish. Natural-colored tilapia were much more preferred than the red. Various marketing strategies for new aquaculture products are discussed.

Introduction

The Kuwait fish catch remained relatively constant from 1975 to 1983 while the Kuwaiti and expatriate populations increased approximately 25% (475,000 to 634,900) and 100% (531,000 to 1,036,800), respectively (Hopkins et al. 1984a; Central Statistical Office, Kuwait 1983). Thus, the per capita fish catch has declined from 5.1 kg in 1975 to only 2.1 kg in 1983. This decline concerns the Kuwaiti government because it increases the reliance of the local population on imported food supplies.

In an effort to increase local supplies of fresh fish, a substantial aquaculture research program was started by the Kuwait Institute for Scientific Research (KISR) in the mid-1970s. This research program used both native and exotic species. A major question facing the program was the acceptability of aquaculture products by the Kuwait populace. As tilapia was an exotic species in Kuwait and was not sold there prior to the start of the program, this question was of particular concern.

Using tilapia grown during the course of experiments at a KISR fisheries station, market tests were started in late 1982 and completed in early 1985. The emphases of the tests were: (1) general observations on consumer acceptance (1982); (2) preferences for size of tilapia, natural-colored (ranging from silvery to almost black) versus reddish-colored tilapia, and sales rates of tilapia in relation to other fresh fish (1983); (3) sales rates of tilapia in different neighborhoods, size and color preferences (1984); (4) sales rates in different neighborhoods and sales rates in relation to other cultured fish (1985).

Methods

All of the market tests were conducted in retail fish stores in Kuwait City. A store in Abdulla Al-Salem, an affluent district inhabited primarily by Kuwaitis, was used in all the tests. During 1984, a second store in a different district was included in the test while in 1985, three additional stores in other districts were used. Three tilapias were used in the tests, natural-colored *Oreochromis aureus* and *O. spilurus* and a red tilapia "hybrid" from Taiwan. They were sorted by size into large (400-700 g), medium (200-300 g) and small (150 g) categories prior to delivery to the stores. As only limited amounts of tilapia were available for the tests (486 kg in 1982, 962 kg in 1983, 824 kg in 1984 and 607 kg in 1985), advertising was restricted to small signs in the stores, a few telephone calls by the store managers to valued customers, and sales talk at the place of purchase.

Tilapia were positioned by price in the prime fish category. To support this positioning, the market strategy emphasized high quality and freshness. Quality was ensured by carefully checking the fish for off-flavors while the fish were still at the fish "farm". If any off-flavors were detected, the fish were held for several more days and were delivered to the store(s) only when off-flavors could no longer be detected. Freshness of the product was ensured by delivering the fish to the stores within 30 min. of removal from the holding tanks. Almost all of the tilapia were still alive upon arrival in the stores and some fish lived for several hours after being placed on ice in display cabinets.

Consumer response was observed by KISR staff stationed in the fish stores during the sales periods. Also, in 1982, 141 questionnaires written in both Arabic and English were distributed to all tilapia buyers. Information regarding the sale rates of other fresh and frozen fish was obtained from the store managers or operators. Additional details of the methods used during the market tests are contained in reports by Hopkins et al. 1984b; Hopkins et al. 1985a; 1985b.

Results and Discussion

The sales of tilapia in 1982 were much more successful than had been expected, with each approximately 50-kg consignment being completely sold in one to two hours. Sales were often made from the delivery trays before the store personnel even had the opportunity to place the tilapia in the display cabinets. The enthusiasm of the Egyptian customers for "their fish" encouraged the Kuwaiti customers to overcome their reluctance to try an unknown fish. Egyptians comprised a majority of the respondents to the questionnaire followed by Kuwaitis and Iraqis (Table 1). A majority of the respondents preferred the medium-sized tilapia, although the Kuwaitis preferred the large tilapia. All buyers indicated that they liked the taste and were willing to buy again if the tilapia was sold live.

In 1983, the percentage of Gulf Arab, presumably Kuwaiti buyers, increased substantially. As in 1982, Kuwaitis preferred the large tilapia and the Egyptians preferred the medium size. The Kuwaitis tended to purchase 5-10 kg of tilapia at one time while the Egyptians usually purchased only 2-5 kg at one time. The sales rate of red tilapia averaged only 15% of the sales rate for natural-colored tilapia. Red tilapia was often mistaken for red snapper which is considered to be a medium- or low-class fish and is not very well liked in the Arabian Gulf area. However, when the customers were told that the fish was a tilapia, they were often willing to purchase at least one fish to try it. The ratio of the average fresh tilapia sales rate during the 1983 sales period to the average sales rate of other fresh fish sold in the store during the month when the test was conducted was 0.72. Imported frozen tilapia was also available in the store at KD0.500/kg versus KD2.250/kg for fresh tilapia. However, only few people purchased the frozen tilapia.

The 1984 sales performance of tilapia at the store in Abdulla Al-Salem (where the previous tests had been conducted) was outstanding while sales at a store located in the Salmiya district were not as successful. The percentage of Kuwaiti buyers remained high in Abdulla Al-Salem but was much lower in Salmiya. As a majority of the customers resided in the same district as the store where they purchased their tilapia, this difference in customers' nationalities could be a reflection of the character of the districts (Abdulla Al-Salem is 70% Kuwaiti while Salmiya is only 10% Kuwaiti). The Kuwaitis again preferred large tilapia but the size preference for tilapia by the Egyptians differed between the two stores, large in Abdulla Al-Salem and medium in Salmiya. Natural-colored tilapia were preferred by all nationalities with only 7-11% of the customers purchasing only red tilapia. However, an increasing number of Kuwaiti customers (40%) purchased both colors of tilapia. The ratio of tilapia sales to other fish sales was 1:1 in Abdulla Al-Salem but only 0.17:1 in Salmiya. The major reason for the poorer response in Salmiya was resistance to high price. Many complaints about high price were heard in Salmiya but not in Abdulla Al-Salem, a much more affluent district. The preference for fresh versus frozen tilapia was again very pronounced with a 10.5:1 ratio of fresh to frozen tilapia sales.

An analysis of sales volumes of other fresh fish during the weeks immediately preceding, during and following the test showed that sales of all fresh fish increased when the live tilapia were present. This indicates that tilapia sales were not at the expense of other fresh fish sales. Instead, the presence of the tilapia attracted customers to the stores.

The 1985 market test clearly demonstrated the potential for competition between fresh cultured tilapia and other fresh cultured fish (porgy, *Acanthopagrus cuvieri* and grouper, *Epinephelus* spp.). Cultured porgy was preferred by Kuwaitis in three of the four stores used in the test (Table 2) while Egyptians preferred tilapia. When all three species were present, over 50% of the cultured fish sold was porgy, 35% was grouper and 15% was tilapia. This species preference was not constant between stores or through time. Tilapia sales were very slow in Mishref and Omariya where almost all of the customers were Kuwaitis unfamiliar with fresh tilapia.

At the Abdulla Al-Salem and Sharq stores, the tilapia sales percentage increased as the test progressed. Most of this increase in tilapia sales was at the expense of grouper sales. Small tilapia could not be sold at the same price as grouper (1,130 g mean weight), porgy (700 g) and large tilapia. However, when the price for small tilapia was halved, the market cleared in a few hours. In Abdulla Al-Salem and Omariya where frozen tilapia are regularly sold, the ratio of live tilapia sales to frozen tilapia sales was 7:1 and 0.7:1, respectively. The lower ratio in Omariya reflected a reluctance by the Kuwaiti customers to buy the fresh tilapia which was being sold for the first time in that district. The frozen tilapia were purchased primarily by working-class Egyptians who indicated they could not afford the fresh tilapia. The ratio of total test fish sales to total sales of other fresh fish ranged from 0.5:1 in Sharq to 2.5:1 in Abdulla Al-Salem.

The market tests conclusively showed that tilapia can be sold as a prime fish if it is sold alive and that large, natural-colored tilapia are more preferred than small or red tilapia. The extent of the potential tilapia market is much more difficult to estimate. In 1983 and 1984, one year after fresh tilapia were introduced into the market, the ratio of fresh tilapia sales to other fresh fish sales ranged from 0.72:1 to 1:1 at the store in Abdulla Al-Salem. Given an annual fresh fish catch of 3,500 t, the potential tilapia market would be 2,500 to 3,500 t/year if: (a) the measured sales ratios can be maintained; (b) there is no competition from cultured grouper and porgy; and (c) the market can absorb the additional fish.

The last assumption can probably be met because doubling the fish supply would increase per capita supplies of fresh fish to about 4 kg/year which is still less than the 5 kg/year level in 1975. But, given the results in Salmiya, it is most doubtful that the high sales prices used in the tests can be maintained if tilapia supplies increased to 2,500-3,500 t/year. Although the current potential for competition from other fresh cultured fish is minimal, economically viable culture systems are currently being developed and may be operational in just a few years. Given these uncertainties, a safe starting point for a commercial tilapia farm would probably be 10% of the 3,500 t/year maximum estimated market. As data on sales are collected, revised estimates of the demand relationship can be made.

These marketing tests illustrate a number of points which should be considered when developing new markets for tilapia and aquaculture products, in general. First, as has been stated by Ray (1978), "Tilapia reputation will be dependent on the image the industry creates." As the Kuwaiti customers were not familiar with tilapia, they had no preconceived notions about the fish. Thus, when the tilapia were carefully presented as prime fish, the customers accepted them as such and were willing to pay prime fish prices.

Second, introducing a new product takes time. Consumers are reluctant to try a new fish. In Abdulla Al-Salem, Kuwaitis comprised only 17% of the tilapia buyers in 1982 but they composed 50% of the buyers in later years. The time required to introduce a new product will substantially increase if the customers have negative preconceived opinions about the product. These prejudices vary greatly throughout the world. For example, the response to red tilapia was initially very negative in Kuwait while red-colored tilapia are often preferred in the Far East. Buyer preferences should be carefully examined before introducing a new product.

Third, only a small number of Kuwaitis purchased tilapia during the 1985 tests in the stores where tilapia had not been sold previously. This indicates that each new market area will have to receive considerable market development before the tilapia are accepted there.

Fourth, the product should be differentiated from potential competitors. We stressed a degree of freshness which only a local aquaculture operation could provide. While locally-cultured fish could still present competition, competition from low-priced frozen imports was minimized.

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Table 1. Nationalities of customers, size preferences and sales rates during 1982, 1983 and 1984 tilapia market tests in Kuwait.

	1982	1983	1984
Nationality of tilapia buyers (%)			
Abdulla Al-Salem store			
Kuwaiti	17	50[a]	44
Egyptian	56	50	38
Other nationalities	27	0	18
Salmiya store			
Kuwaiti	–	–	22
Egyptian	–	–	60
Other nationalities	–	–	18
Size preference based on plurality			
Abdulla Al-Salem store			
Kuwaiti	large	large	large
Egyptian	medium	medium	large
Other nationalities	medium	–	large
Salmiya store			
Kuwaiti	–	–	large
Egyptian	–	–	medium
Other nationalities	–	–	large
Sales ratios			
Natural-colored: red tilapia	–	7:1	–
All tilapia: all other fresh fish	–	0.72:1	1:1
Fresh tilapia: frozen tilapia	–	–	10.5:1

[a] Gulf Arab, presumably Kuwaiti.

Table 2. Composition of cultured fish sales in Kuwait during 1985 market test.

Store location and fish type	% cultured fish sold by date[1]					
	March					5-day duration
	3	4	5	6	7	
Abdulla Al-Salem[2]						
Tilapia	11	4	4	29	38	18
Grouper	45	44	36	7	24	32
Porgy	44	52	60	64	38	50
Mishref[3]						
Tilapia	–	7	–	4	–	7
Grouper	–	55	–	21	–	43
Porgy	–	38	–	75	–	50
Omariya[4]						
Tilapia	–	9	12	6	9	9
Grouper	–	18	39	36	24	30
Porgy	–	73	49	58	67	61
Sharq[5]						
Tilapia	–	18	–	15	27	20
Grouper	–	52	–	23	26	37
Porgy	–	30	–	62	47	43

[1] % when all 3 types of fish were present with days when this condition was not met, shown as "–"

[2] The only district in this test where fresh tilapia was previously sold, Kuwaitis comprising 70% of residents.

[3] Kuwaitis comprised 57% of residents.

[4] Kuwaitis comprised 69% of residents.

[5] Kuwaitis comprised 9% of residents.

Effects of Storage on the Microbial Quality of Slipper Oysters, *Crassostrea iredalei**

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Abstract

The effects of storage on the microbial quality of slipper oysters, *Crassostrea iredalei*, were examined. Oysters were stored at room temperature (24°C), under a blanket of ice (3-4 C), chilled (4-C) and frozen (-25 C) until they spoiled. The shelf life of oysters stored at room temperature was only two days. Oysters held under a blanket of ice had a shelf life of 14 days and chilled oysters, 22 days. Frozen oysters remained in good condition over the 64 day storage period.

The initial total aerobic bacterial count of oysters was 10^5cfu/g. Counts for frozen oysters decreased by 1 log (10^4) while counts for oysters stored at other temperatures increased by 2-4 log (10^7-10^9). Bacterial typing of 50 randomly-picked colonies made every four days showed *Pseudomonas* to be the predominant spoilage organism. Total and fecal coliform counts did not increase even for oysters held at room temperature. Typical *Staphylococcus aureaus* colonies were isolated but were shown to be non-pathogenic by the coagulase test. Analyses for the presence of other organisms of public health concern revealed that *Salmonella, Shigella, Vibrio cholerae, V. parahaemolyticus*, Lactose + Vibrios *(V. vulnificus)* and fecal streptococci were present in very low or undectectable levels. Thus, hazards or risks associated with these organisms may be considered minimal.

Introduction

Oysters are transported, stored and marketed live to preserve their unique flavor and texture. During storage and subsequent phases of marketing, quality changes occur even when the shellfish are still alive (Warwick 1985). The rate and extent of these changes depend on the temperature at which the oysters are handled and stored (Boyd and Wilson 1978). One of the major problems facing the industry relates to postharvest handling: how to prevent or minimize quality changes and how to lengthen the short shelf life of the product. The other problems relate to public health safety, oysters being grown usually in areas subject to pollution from sewage and farm run-off. There is growing concern that these shellfish may pose a risk or hazard to the consuming public because many people are used to eating oysters raw or partially cooked (Chai et al. 1984; Fernandez 1985).

Appropriate methods of shelf life extension can be developed only if the postharvest quality changes are known and well understood. Thus, this study determined the effects of storage on the microbial quality of the slipper oysters, *Crassostrea iredalei*. It addressed the two major problems of the industry, the quality (spoilage organisms) and the safety (public health organisms) of oysters.

Materials and Methods

Samples were obtained from Lutod-Lutod, Capiz, Philippines, and transported live to the SEAFDEC laboratory in Tigbauan, Iloilo, Philippines, where they were washed and cleaned of mud and dirt. The oysters were divided into lots and then stored on shellstock at room temperature (24°C), under blanket of ice (3-4°C), chilled in the refrigerator (4°C), and frozen (-25°C) up to the time they spoiled. Oysters stored under a blanket of ice were placed in a styrofoam box. To prevent them from having direct contact with the ice, a layer of clean sack cloth was placed over them before they were covered with ice. Water from melting ice was drained regularly, and the ice continuously replenished to maintain the temperature of 3-4°C. Sampling for bacteriological analyses was made at day 0, then daily for oyters held at 24°C, every two days both for chilled and iced oysters, and every four days for frozen oysters.

Microbiological examinations for total aerobic plate count (20°C), total and fecal coliforms, fecal streptococci, *Staphylococcus aureus, Salmonella, Shigella, Vibrio cholerae, V. parahaemolyticus* and Lactose + *vibrios* were performed according to the procedures described in the Bacteriological Analytical Manual for Foods (FDA 1976) and the Compendium of Methods for the Microbiological Examination of Foods (Speck 1976). Where applicable, initial dilution was made on a weight basis recommended by Cook and Dicharry (1984), using 1:1 sample diluent. This was then homogenized in a Stomacher-Lab-Blender (Seward Medical Lab House, Balckfriars Road, London) for two minutes. From this homogenate, 20 g was obtained and added to 80 ml of diluent to make the 1:10 dilution. Subsequent higher dilutions were made from the 1:10 dilution on a volume basis.

Representative colony types (50) were randomly picked from countable plates every four days. Isolates were identified up to the genus level with diagnostic procedures and identification schemes described by Vanderzant and Nickelson (1969), Lewis (1973) and Buchanan and Gibbons (1974).

Chemical indicators of spoilage, total volatile nitrogen (TVN) and trimethylamine (TMA), were also determined each sampling time with the modified Conway diffusion methods of Cobb et al. (1973). Saturated $Na_3PO_4.KOH$ was used as releasing agent and 3.1% boric acid with Conway indicator as trapping agent. Samples were allowed to diffuse for two hours before back titrating with 0.02 N H_2SO_4. The same procedure was used for TMA determination except that 0.5 ml of 40% formaldehyde was added to the sample prior to reaction with releasing agent.

Results and Discussion

Bacterial flora. Table 1 shows that fresh oysters had an initial aerobic plate count (APC) of 10^5 cfu/g, increasing to 10^7-10^9/g, during spoilage, consistent with results of other studies (Liston 1980). Depending on the water temperature where they are grown, oysters may carry a resident population of bacteria ranging from 10^4 to 10^6 cfu/g tissue. During spoilage, the population may reach 10^7 cfu/g or higher, with *Pseudomonas* as the major spoilage bacteria. Vanderzant et al. (1973) and Hood et al. (1983) found that the initial bacterial count of *Crassostrea virginica* (Eastern oysters) ranged from 10^5 to 10^7/g, while (Vasconcelos and Lee 1972) found that of *C. gigas* (Pacific oysters) to be 10^4/g. An initial count of only 10^3/g was observed by *C. commercialis* (Sydney rock oysters) by Eyles and Davey (1984). Counts for frozen oysters showed a decrease of log 10^4 during the 64-day storage.

The initial microflora observed in oysters (Table 2) were predominantly (57-72%) gram negative rods consisting of *Pseudomonas*, *Vibrio*, *Aeromonas*, *Flavobacterium*, *Acinetobacter/Moraxella* and enterobacteriaceae. Gram positive populations of *Micrococcus*, *Bacillus*, *Staphylococcus*, *Streptococcus* and coryneform bacteria were also present (28-43%). These results are in agreement with the findings of Colwell and Liston (1960), Vanderzant et al. (1973) and Vasconcelos and Lee (1972). Except in chilled oysters where *Micrococcus* dominated, *Pseudomonas* was likewise found to be the major spoilage bacteria (Table 2). Studies have consistently shown that bacterial load during spoilage of fishery products is usually dominated by the gram negative, aerobic, psychrotrophs, particularly the *Pseudomonas* group. This indicates that spoilage is more closely related with the growth of specific type of bacteria than with the total bacterial load present. Pseudomonas was found to be more closely correlated to the cold storage (ice/chill) keeping time of fishery products (Martin et al.1978).

Indicator organisms. Fresh or frozen oysters are considered acceptable if the fecal coliform (FC) counts of 10% of the sample do not exceed 2.3 MPN/g (Speck 1976). The initial MPN counts for total (TC) (240-1,100/g) and FC (110-400/g) of the oysters used in this study (Table 1) were higher than the acceptable level, which might be a reflection of the quality of growing waters and the climate (rainfall). Samples were obtained on November 1984, shortly after a major typhoon (Undang) hit the area. A study by Qadri et al. (1975) showed that heavy rainfall may vary from no effect to marked direct effect on oyster pollution. A significant increase in FC in *C. gigas* was observed by Brown and McMeekin (1977) after heavy rainfall in the area where the oysters were collected. Table 1 also shows that the TC and FC counts did not increase even in oysters held at room temperature. Qadri et al. (1975) suggested that there might be some antimicrobial factors present in the oysters which inhibit the multiplication of coliforms during storage. This implies that cold storage does not increase the risk associated with these organisms.

Public health organisms. *Staphylococcus* counts in oysters were initially 10^4 cfu/g (Table 1). Counts for frozen oysters decreased by 1 log (10^3) after 20 days, while samples held under a blanket of ice, and chilled increased by 1 log (10^5) during storage. There was no significant increase in counts in oysters held at room temperature for two days. Typical *S. aureus* colonies were isolated but were found to be non-pathogenic by the coagulase test.

Isolation for other public health organisms, *Salmonella*, *Shigella*, fecal streptococci, *V. cholerae*, *V. parahaemolyticus* and Lactose + *vibrios* were also performed at day 0. Of the 50 presumptive *Salmonella* colonies recovered from bismuth sulfite (BS), brilliant green (BG), and salmonella-shigella (SS) agar, none was confirmed as *Salmonella*. Oyster samples were also negative for fecal streptococci and *Shigella*. Typical Vibrio colonies from thiosulfate citrate bile sucrose (TCBS) and gelatin agar were found to be non-pathogenic. Reports of increasing incidence of diseases associated with the consumption of raw oysters have attributed the causes to these public health organisms, particularly *Salmonella* and the *Vibrios* (Liston 1980). Since oysters are filter feeders, they are able to concentrate microorganisms, including pathogens, in their tissues. There is concern that these organisms may pose a potential hazard when they survive or increase during normal handling and storage. *Salmonella* has been observed to survive for 10-14 days in oysters during cold storage (Hood et al. 1983; Fraiser et al. 1984). Hood et al. (1983) further observed the increase in levels of *V. cholerae* and Lactose + *Vibrios* in cold-stored oysters. While these organisms were not detected in the oyster samples studied, future investigations should evaluate the changes and behavior during storage whenever these pathogens are found present.

Total volatile nitrogen and trimethylamine. At present, no simple and reliable tests for oyster quality are available. Among the chemical indicators of quality developed for fish and shrimp, TVN and TMA have been suggested as chemical indicators of quality of oysters. The TVN contents (in mg N/100 g tissue) of oysters stored at different temperatures are shown in Fig. 1. High increases were observed in oysters held under ice (from 4.67 to 49.76 after 12 days) and chilled (from 3.93 to 37.37 after 22 days). Oysters stored at room temperature had TVN value of only 5.9 after two days. After freezing for 64 days, TVN value of oysters remained low at 5.9, with highest value of 15.4 at day 48. The suggested TVN values for evaluating the quality of fish are: 12 mg for fresh fish; 12-20 mg for good quality and slightly decomposed; 20-25 mg for edible and acceptable; and 25 mg for decomposed and unacceptable/inedible fish (Stansby 1963). Based on these values, oysters stored at room temperature would have been considered still fresh after 2 days, while oysters held under ice and chilled would have been considered spoiled by day 10 and 20, respectively. Frozen oysters never reached values of 16 mg indicating that they remained in good condition during storage of 64 days.

The TMA values (in mg N/100 g tissue) of oysters stored at room temperature progressively increased for two days as shown in Fig. 2. However, there was no particular trend in TMA values in oysters at other temperatures. Cobb et al. (1973) suggested a TMA value of 5 mg/100 g as the limit of acceptability in fresh shrimp. Using this value, the oysters would not have been rejected even when they were obviously spoiled.

Values obtained both for TVN and TMA suggest that these indices might not be suitable in evaluating the quality of oysters. This was also pointed out by Lartigue et al. (1960) and Liuzzo et al. (1975).

Shelf life of oysters. The storage life of oysters under different storage conditions is shown in Table 3. Oysters were considered spoiled when the APC reached 10^7-10^9 cfu/g and the odor was objectionable. Oysters held at room temperature spoiled in two days, under a blanket of ice in 14 days and chilled by day 22. Frozen oysters remained in good condition over the 64-day storage period. While chilled oysters were still acceptable by day 20, based on the rejection criteria mentioned, the oyster meats were already dehydrated and dried when they were rejected (day 22).

The most effective and practical storage condition observed was under a blanket of ice. The oyster meat remained moist and wet throughout storage. The ice-blanket method was originally developed for mussels and scallop (Boyd and Wilson 1978; Brooks and Harvie 1981; Thrower 1983; Warwick 1985). The 14-day shelf life of oysters held under a blanket of ice closely parallels the results obtained for mussels by Boyd and Wilson (1978), Brooks and Harvie (1981) and Warwick (1985) who reported that mussels remained in good condition in 9-14 days.

Provided that clean ice from potable water is used and the melt water drained regularly, storage of oysters under a blanket of ice may prove to have practical and economic applications. It can extend the keeping quality of oysters up to 14 days, thereby extending its marketing possibilities.

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Table 1. Total aerobic plate counts (APC), total coliforms (TC), fecal coliforms (FC) *Staphylococcus* counts of oysters at different temperatures.

Storage temperature	Day	APC (cfu/g)	TC (MPN/g)	FC (MPN/g)	*Staphylococcus* (cfu/g)
A Room temperature 24°C	0	1.4×10^5	1,100	100	1.5×10^4
	1	2.0×10^6	1,100	110	7.0×10^4
	2	7.8×10^7	1,100	110	2.0×10^4
B Under blanket of ice 3-4°C	0	5.5×10^5	1,100	460	1.8×10^4
	2	2.0×10^6	1,100	240	3.5×10^4
	4	5.4×10^5	460	460	2.0×10^4
	8	3.9×10^5	—	240	4.0×10^5
	10	7.3×10^7	—	—	3.6×10^5
	12	7.8×10^8	460	150	—
	14	$> 10^8$	—	—	9.8×10^5
C Chilled 4°C	0	5.7×10^5	1,100	110	3.0×10^4
	2	8.2×10^5	460	240	1.6×10^3
	4	7.5×10^7	240	240	—
	6	3.9×10^6	240	240	3.6×10^4
	8	1.9×10^5	—	—	4.5×10^4
	10	7.2×10^5	930	—	8.9×10^4
	12	1.9×10^7	240	240	1.0×10^4
	16	1.1×10^8	460	—	4.0×10^4
	22	3.9×10^9	23	23	3.4×10^4
D Frozen –25°C	0	1.7×10^5	240	110	1.3×10^4
	4	1.7×10^5	—	—	4.4×10^4
	8	1.2×10^5	430	240	1.8×10^4
	12	5.3×10^5	210	210	3.0×10^3
	16	5.0×10^4	29	240	1.4×10^4
	20	1.4×10^5	—	—	1.0×10^3
	24	4.2×10^4	93	43	4.0×10^3
	28	2.7×10^4	—	—	2.0×10^3
	32	2.1×10^4	93	93	—
	36	7.9×10^4	—	—	—
	40	3.1×10^4	9.1	3.6	—
	44	1.0×10^5	21	15	—
	48	1.8×10^4	9.1	3.6	—
	52	5.2×10^4	23	23	—
	56	1.7×10^4	11	3.6	—
	64	3.7×10^4	9.1	9.1	—

Table 2. Percentage distribution of the microbial flora of oysters stored at different temperatures.

Storage temperature	Day	Pseudomonas	Vibrio	Aeromonas	Flavobacterium	Enterobacteriaceae	Acinetobacter/ Moraxella	Coryneforms	Micrococcus	Bacillus	Streptococcus	Staphylococcus
A Room temperature 24°C	0	10.3	6.9	13.8	6.9	13.8	10.3		6.9	6.9	3.4	13.8
	2	30.4	19.6	13.0	6.5	8.7	4.3	10.8	6.5			
B Under blanket of ice 3-4°C	0	12.5	2.5	22.5		12.5	7.5	37.5	2.5	2.5		
	4	20.0		20.0	10.0	10.0						40.0
	8	66.6				33.3						
	12	71.4						28.6				
	14	68.4		10.5		5.3		10.5	5.3			
C Chilled 4°C	0	7.7	18.0	38.5		7.7		23.1		5.0		
	4	12.5		12.5		6.3		31.2				37.5
	8	28.6	14.3	21.4		14.3		7.1		7.1		7.1
	12		14.3		14.3			28.6	14.3		28.6	
	22								100.0			
D Frozen –25°C	0	36.8	7.9	13.2	7.9	5.3		26.3		2.6		
	4	23.5		17.6	5.9	5.9		29.4				17.6
	8	14.3		21.4	14.3	28.6	7.1	14.3				
	12	8.3		33.3		8.3		8.3		25.0		16.7
	16	36.3			9.1	18.2			18.2			18.2
	20	20.0				6.6		46.7		26.7		
	24	16.6							16.6	66.7		
	28	25.0						16.6	41.7	16.6		
	32	18.2						18.2	36.4	27.3		
	36	6.7		6.7				13.3	13.3	46.7		13.3
	40	25.0						43.8		18.8		6.2
	44	66.7						6.7	13.3	13.3		
	48	26.3					10.5	52.6	5.3	5.3		
	52	21.0		5.3		21.8		26.3	10.5	10.5		
	56	31.7						26.3	10.5	21.0		
	64	30.8		15.4	20.5	5.1		10.2	5.1	7.7		5.1

Table 3. Shelf life of oysters at different storage conditions.

Storage temperature (°C)	Shelf-life (days)
Room temperature 24	2
Under blanket of ice 3-4	14
Chilled 4	22
Frozen –25	64

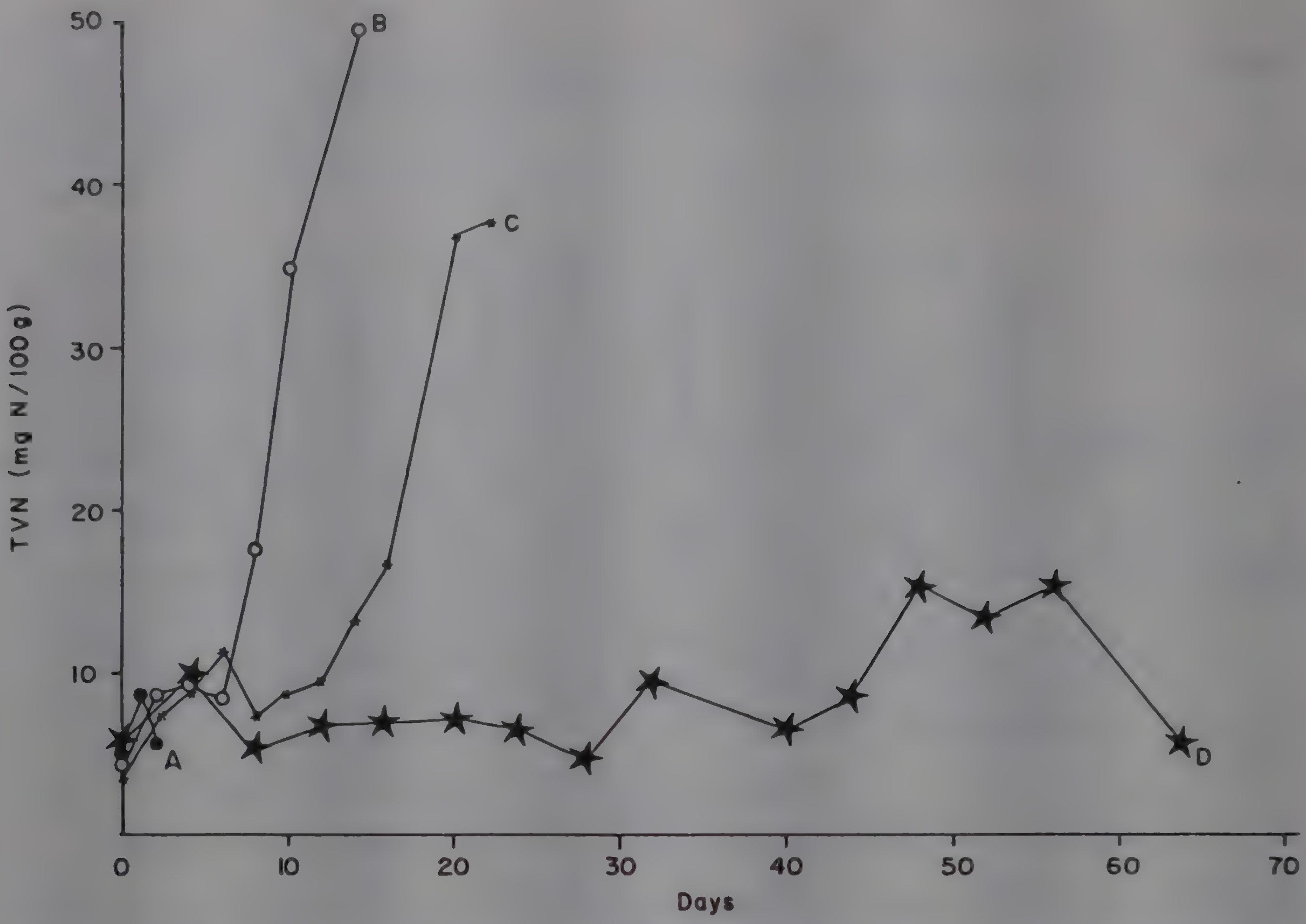

Fig. 1. Total Volatile Nitrogen (TVN) value in oysters during storage at different temperatures (A = room temperature, 24 C; B = under blanket of ice, 3-4 C; C = chilled, 4 C; D = frozen, –25 C).

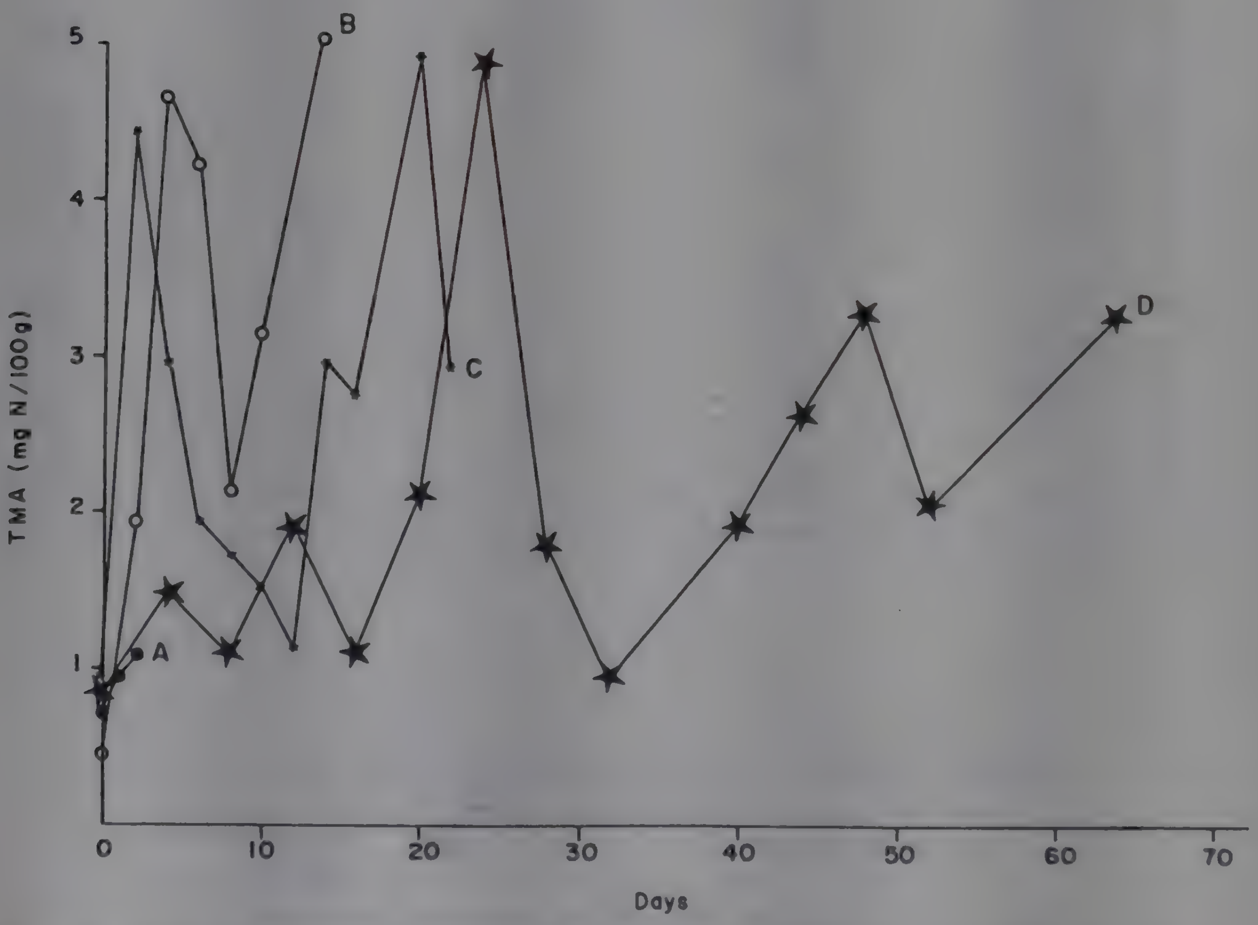

Fig. 2. Trimethylamine (TMA) values in oysters during storage at different temperatures. (A = room temperature, 24 C; B = under blanket of ice, 3-4 C; C = chilled, 4 C; D = frozen, –25 C).

The Microbiology of Cooked Rice-Minced Fish Fermentation

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Abstract

Cooked rice and minced round scad (*Decapterus macrosoma* Bleeker) mixture was fermented in an anaerobic system. Commercial shrimp paste was used as inoculum to ensure that lactic acid bacteria found in traditional fermented fish were present in the mixture. Fermentation was carried out for 25 days at 30°C. The development of the microbial population and the physicochemical properties were observed. The lactic acid bacteria isolated from 12 sampling periods were characterized and screened for their starch hydrolyzing properties. The mixture appeared and smelled like the traditional product. The pH dropped from 6 to 3.4 in 48 hours. Most of the isolates were lactic acid bacteria. Two groups of bacteria similar to *Lactobacillus plantarum* and to *Lactobacillus casei subsp. pseudoplantarum* dominated throughout. Both showed varying degrees of starch hydrolysis brought about by the action of these organisms.

Introduction

Fish preservation in rural areas, especially during inclement weather, is always a problem. Lack of ice and refrigerating units on board small fishing boats lead to the spoilage of much of the catch before landing. Preservation is mainly by drying, salting, smoking and biological fermentation. In inclement weather, only fermentation and salting are applicable. Salted fish has limited consumption because of high salt content. Currently, there is an interest in the use of bacterial fermentation not only for food preservation but also for fish silage.

There are various types of rice-fish or shrimp fermented sauces. Most of the studies conducted, however, are on the development and understanding of the process (Orillo and Pederson 1968; Arroyo et al. 1978; Guevara et al. 1978; Vatana and Del Rosario 1983) and only a few deal with the enumeration and identification of the organisms present (Solidum 1979; Uyenco and Ajon 1982). Slow acid production results in poor quality product and greater health risk. Success in fermentation lies in the knowledge of the microorganisms involved, in this case lactic acid bacteria.

The ability of lactic acid bacteria to initiate fermentation of rice-shrimp mixture has not been clearly demonstrated. In rice-prawn sauces, no sugar or amylase is added and yet the mixture ferments successfully. It is strongly suspected that lactic acid bacteria are directly involved in the breakdown of starch, resulting in the formation of fermentable sugar.

This study aims to investigate the nature and role of lactic acid bacteria in rice-fish fermentation, using the commercially important pelagic round scad *Decapterus macrosoma*.

Materials and Methods

Fig. 1 shows the preparation of the rice-fish mixture. The mixture was packed in 26 bottles (150 ml capacity) and 5 g solid CO_2 was placed inside to displace the air before fitting the fermentation lock. The bottles were incubated at 30°C for 25 days. *Balao-balao*, fermented shrimp paste, was used to ensure presence of lactic acid bacteria.

Microbial counts, pH values and titrable acid were determined. Two bottles were examined after every 13 and 30 hours, 2-8, 11 and 25 days of incubation.

The following media were used to enumerate the following: APT (Difco) and MRS (Oxoid) agar or broth for viable counts; TGYE-$CaCO_3$-Carbonyl-Methyl-Cellulose as modified from Keston and Rosenberg (1967) for acid producing organisms; and malt extract agar (Oxoid) for yeasts. An appropriate dilution in 0.1% peptone water was spread on agar plates, overlaid with similar medium and incubated at 30°C for 48 hours. Representative colonies (30) were randomly picked from one APT agar plate using a Harrison's disc (Harrigan and McCance 1976) as guide. The colonies were grown in

APT broth and purified, after which they were stored in yeast-glucose-chalk-litmus-milk.

Morphological and biochemical test procedures followed were those described by Sharpe et al. (1966) unless otherwise stated. APT medium was used for general cultivation since some isolates showed long lag phase in MRS medium; however, when thick cell suspensions were required the latter was used because of its stronger buffering capacity.

Gram reaction, catalase formation and ability to grow in deep shake cultures (Stanier et al. 1976) were used to screen lactic acid bacteria. Gas production from glucose, deamination of arginine by lactobacilli (Briggs 1953) and streptococci (Abdel Malek and Gibson 1948) and dextran production (Garvie 1960) were examined.

Lactic acid bacteria were further characterized by their ability to grow at 10, 15, 45 and 48°C; survive at 60°C for 30 min.; and grow at various pH values and NaCl concentrations. The last two tests were conducted in Microtitre plates developed principally for tests requiring several replicates (Mendoza, unpublished data). Hydrolysis of starch (Dunican and Seeley 1962), gelatin, fat and esculin (Naylor and Sharpe 1958) were also examined. Fermentation of sugar followed the method of de Mann et al. (1960) except for the addition of 0.1% Nobel agar (Difco) as thickening agent in the MRS basal medium. The medium was dispensed mechanically into Microtitre plates (0.24 ml/well). A 48-hour culture in MRS broth was harvested, washed and resuspended in MRS basal wash (omitting chlorophenol red and agar). A Pasteur pipette drop of this suspension was inoculated into each Microtitre well. The plates were incubated at 30°C and examined after 24 and 48 hours. Acid formation was indicated by a change in red to yellow.

Twenty-one suspected lactic acid bacteria samples grown in APT-starch were dispensed in test tubes and autoclaved at 121°C for 15 min. before sterile glucose (0.05-1% w/v) was added. The tubes were inoculated with a drop of 48-hour culture in APT broth and incubated for five days. The hydrolysis of starch and acid production was shown by the change in color with iodine solution and chlorophenol red.

Results and Discussion

The rice and minced meat were cream and reddish brown, respectively. During fermentation the mixture became moist and gas was evolved. After seven days the fishy odor disappeared and was replaced by a sour-cheese like odor similar to the traditional products.

The average total viable organisms increased rapidly during the first two days and decreased gradually until they became stable on the 6th day (Fig. 2). The counts in MRSA and APTA were similar, an indication that MRSA did not inhibit the microbial population. A selection of 360 isolates was taken at random from APTA plates and subjected to a characterization process.

The acid producers in TGYE-CMC-$CaCO_3$ agar plates increased at 10^8 organisms/g in 2 days (Fig. 3). Nonacid producers also incresed but were rapidly overtaken by the acid producers on the fourth day. All colonies considered as nonacid producers in TGYE-CMC-$CaCO_3$ agar plates were actually weak acid producers. This was confirmed by their ability to produce acid after a three-day culture in APT soft agar tubes. Yeasts appeared on the 8th day which might be due to the leakage in some fermentation locks.

The pH decreased from 6 to 3.4 in two days (Fig. 4), an indication of rapid fermentation. Titrable acid as lactic acid increased gradually but on prolonged incubation, higher values (2.75% after 25 days and 4.2% after 60 days) without corresponding decrease in pH were observed. To some extent this was attributed to the buffering capacity of the mixture.

Isolates (total of 360) were obtained from 12 samples distributed over 25 days. The isolates were presumptive lactic acid bacteria (98%) and yeasts (2%). The lactic acid bacteria were divided into six groups based mainly on the production of CO_2 from glucose, fermentation of carbohydrates, growth at 10°C and failure to grow at 45°C. No obligate aerobic bacteria were isolated, presumably due to the displacement of air and the inhibitory effect of CO_2.

The strains showed many similar characteristics. They were gram positive, catalase negative, produced acid from glucose and failed to produce dextran from sucrose. All groups showed ability to grow in deep shake cultures; at 10°C to 37°C but not at 45°C; and in 4 to 6.5% NaCl, but weak at 10% and negative at 18.5% NaCl concentration. Growth was also initiated at pH 3.5 to 8.75 but failed to survive a 30 min. heat treatment at 60°C. All strains failed to hydrolyze gelatin and tributyrin but showed various degrees of starch hydrolysis. They all fermented fructose, galactose, lactose and maltose. Other differentiating properties are listed in Tables 1 and 2. The differences between strains are mostly in their ability to ferment the different sugars. The strains showed typical characteristics which cannot be identified with known species.

The development of the microflora during fermentation is shown in Fig. 5. Lactobacilli were isolated throughout the fermentation processs and the product fermented successfully even in the absence of other types of lactic acid bacteria. Groups I and II which are similar to *Lactobacillus casei subsp. pesudoplantarum* and *Lactobacillus plantarum*, respectively, were present throughout the incubation period. Because of this and especially their presence during the initial stage of fermentation, they are believed to have played an important role in fermentation. Coccal rods (Group III) were isolated during the first three days but disappeared altogether in the later stages of fermentation. This might be an indication of their low acid tolerance. The heterofermentative groups appeared to be less dominant.

One distinct characteristic shown by these isolates was their ability to hydrolyze starch and convert it to assimilable form. This was not reported in previous studies on fermented fish. Most of the isolates hydrolyzed starch under the colonies except 12 isolates of Group II which showed clear zones around the colonies (Table 1) in APT-starch agar. Further examination of this property in liquid medium showed that strains in Group II and half of Group III can hydrolyze the starch even in simple media containing starch and NaCl only. Upon the addition of 0.05% glucose to the same medium, the other half of the isolates in Group III hydrolyzed the starch. Finally, isolates in Group I showed this property only when grown in complex medium (APT) containing 2% starch and 1% glucose.

This study deviates from the general impression that lactic acid bacteria are not capable of hydrolyzing starch into assimilable form. These strains might be unreported species or just strains that could have developed characteristics to survive in their present environment that were not shown before.

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Table 1. Properties where lactobacilli isolates showed differences.[1]

Properties	Groups I	 II	 III	 IV	 V	 VI
Shape	rod	rod	coccal rod	rod	rod	rod
NH_3, arginine	—	—	—	D	+	+
CO_2 from glucose	—	—	—	—	+	+
Hydrolysis of						
starch	sl	+(12), sl	sl	sl	sl	sl
esculin	+	+	—	—	—	—
Total isolated[2]	273	38	22	4	14	4

[1] The results of all other properties examined were similar.

[2] Number isolated out of 360; D, some positive; sl, clearing under the colonies only.

Table 2. A summary of the carbohydrate fermentation results in Microtitre plates.

Carbohydrates	Groups I	 II	 III	 IV	 V	 VI
Arabinose	—	+	+	+	+	+
Ribose	D	+	—	D	+	—
Xylose	—	D	—	—	—	—
Mannose	+	+	+	D	—	W
Celliobiose	+	+	+	+	—	—
Melibiose	—	+	+	—	+	—
Sucrose	+	+	W	+	—	—
Melizitose	+	D	D	+	—	—
Raffinose	—	+	—	—	D	D
Mannitol	+	+	+	D	+	W
Sorbitol	+	+	W	D	+	—
Rhamnose	—	D	—	D	—	—
Amygdalin	D	+	+	+	—	—
Salicin	D	+	—	D	—	—
Gluconate	+	D	W	—	+	+

D, some negative; W, week reactions; +, strong fermentation; —, no fermentation. All groups fermented fructose, galactose, lactose and maltose.

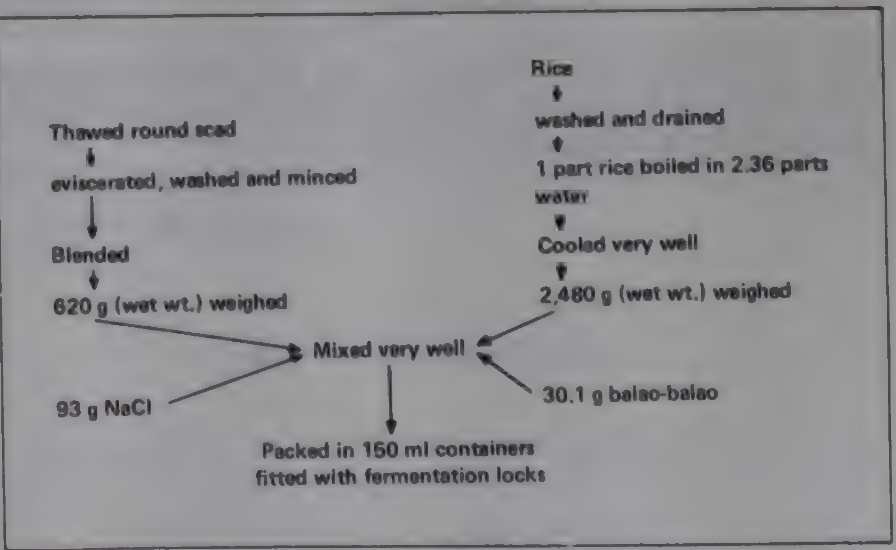
Rice
washed and drained
1 part rice boiled in 2.36 parts water
Cooled very well
2,480 g (wet wt.) weighed
Thawed round scad
eviscerated, washed and minced
Blended
620 g (wet wt.) weighed
Mixed very well
93 g NaCl
30.1 g balao-balao
Packed in 150 ml containers fitted with fermentation locks

Fig. 1. Preparation of rice and minced round scad mixture.

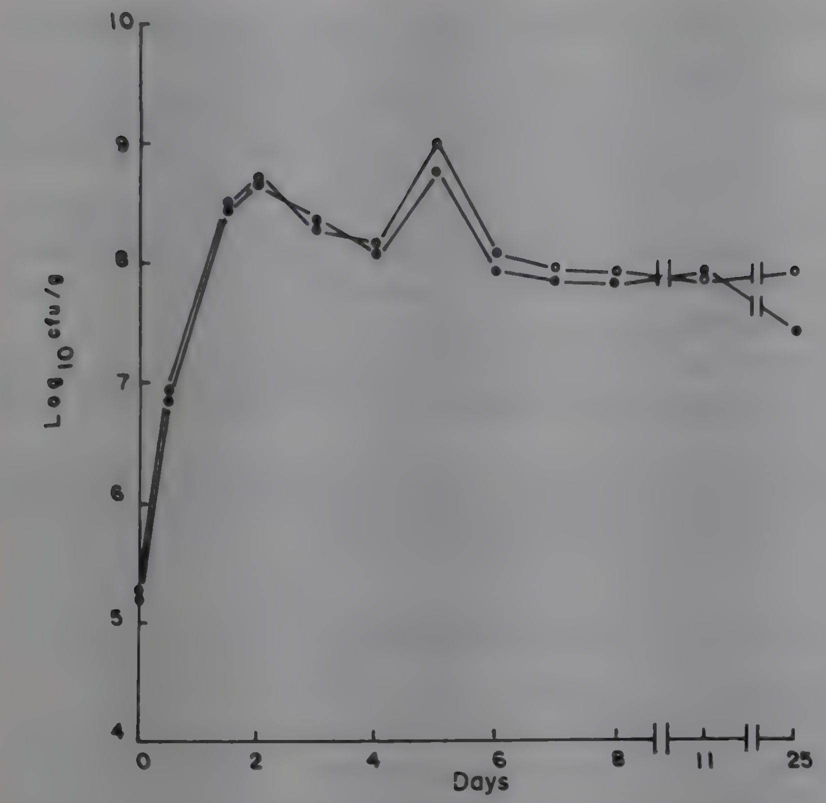

Fig. 2. Changes in the microbial counts of fermenting rice-minced fish mixture.

●, APT agar; O, MRS agar

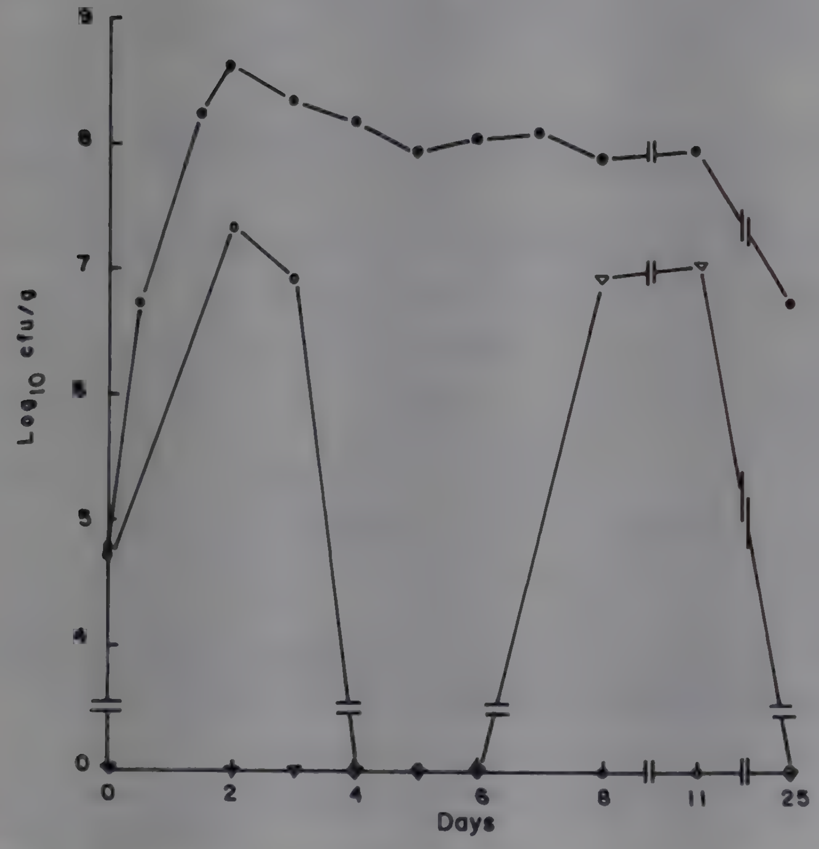

Fig. 3. Changes in the number of acid and nonacid producers, and yeasts in fermenting rice-minced fish mixture. ●, acid producers; ○, nonacid producers; △, yeasts.

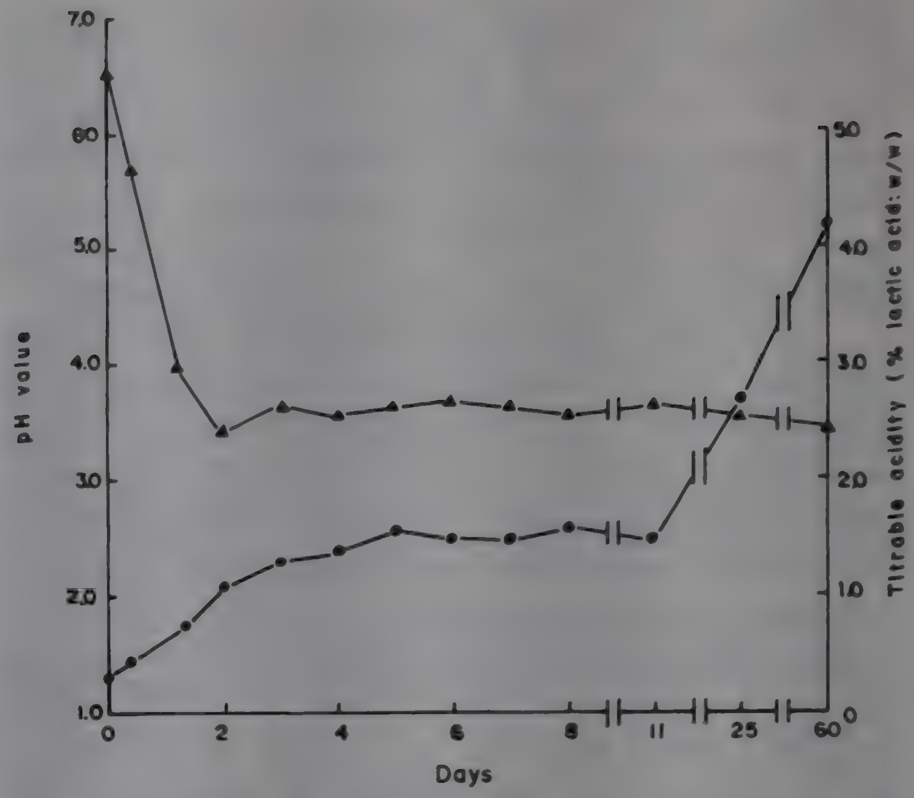

Fig. 4. Changes in the titrable acid as lactic acid and pH during the fermentation of cooked rice and minced roundscad in an anaerobic system. ●, % titrable acid; ▲, pH values.

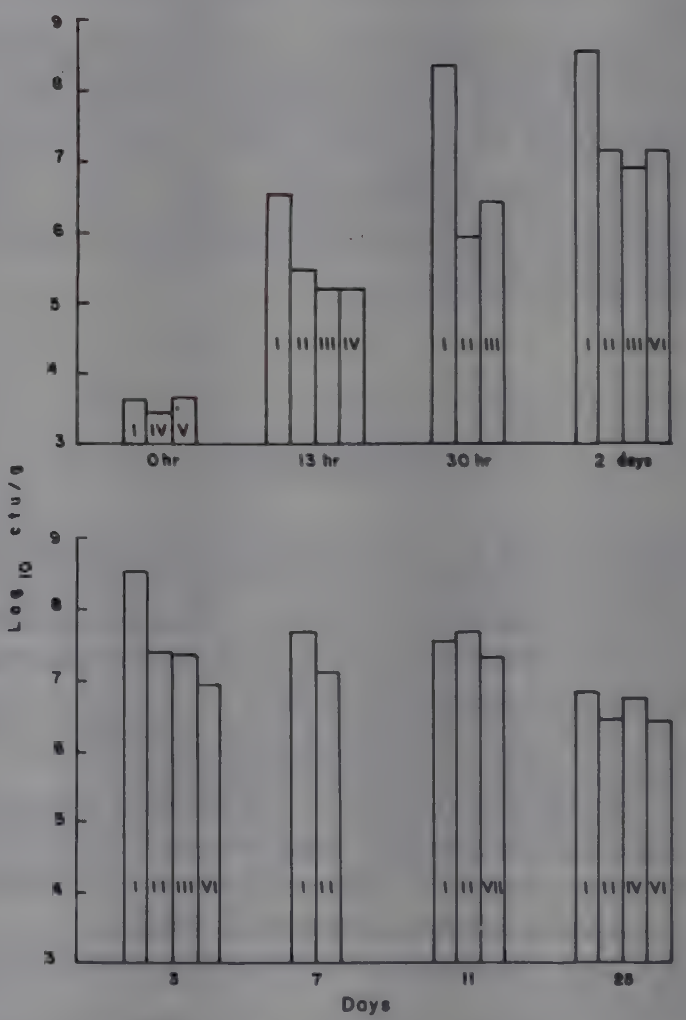

Fig. 5. Development of the microflora of rice and minced roundscad during fermentation. Homofermentative Lactobacilli: I, similar to *Lactobacillus casei* subsp. *pseudoplantarum;* II, similar to *Lactobacillus plantarum;* III, unidentified coccal rods; IV, unidentified rods. Heterofermentative Lactobacilli: V, closely resembles *Lactobacillus brevis;* VI, unidentified. Fungi: VII, unidentified yeasts.

The Sanitary Quality of Four Philippine Oyster-Growing Areas*

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Abstract

Microbial analysis of estuarine waters in four sites in Pangasinan province, Philippines, showed them to be, by foreign standards, prohibited and unfavorable areas for oyster-growing because the concentration of coliforms and *Escherichia coli* generally far exceeded the microbiological limits set by the United States, Italy and France for approved and satisfactory oyster-growing areas. This study underscores the need to depurate the oysters harvested from these areas prior to raw consumption, and to identify clean, although non-traditional, sites for oyster growing, if oysters are to be pursued as products for export.

Introduction

The sanitary quality of oyster-growing areas is assessed by analyzing for fecal indicator organisms, *Escherichia coli*, although coliform analyses are successfully used in some countries. In the United States, growing areas are classified as approved, conditionally approved and prohibited. Approved areas are those with a coliform Most Probable Number (MPN) of $<$ 70/100 ml; moreover, not more than 10% of samples taken during the most unfavorable hydrographic and pollution conditions must have an MPN of $>$ 230/100 ml. Prohibited areas are those that do not comply with the above criteria. Conditional areas are those which receive intermittent pollution from one or more sources. In Italy, approved areas are those with *E. coli* MPN of $\leq$2/100 ml in 90% of samples taken in one year; not more than 10% of samples taken in one year must contain $>$ 6 *E. coli*/100 ml. Source waters of oysters in France are categorized as class I (satisfactory, no *E. coli*); class II (acceptable, 1-60 *E. coli*/100 ml); class III (suspicious, 60-120 *E. coli*/100 ml) and class IV (unfavorable, over 120 *E. coli*/100 ml) (Wood 1976; Hunt 1979).

Oyster-growing areas in the Philippines are of unknown sanitary quality, hence, not classified. It is known, however, that oysters are grown in polluted waters. Apparently, there is little or no sanitary regulation to protect public health from the possible hazards of consuming contaminated oysters. Since oysters are an important export commodity, cultivation areas should be clean, considering that most potential buyers of oysters impose strict requirements with regard to cleanliness of the source waters.

This study determines the sanitary quality of four traditional oyster-growing areas in Pangasinan: Dawel in Dagupan City, Salapingao and Gayaman in Binmaley and Nibaliw Central in San Fabian. Pangasinan is one of the major producers of cupped oysters *(Crassostrea)* in Luzon.

Materials and Methods

The sources of pollution in each study area were identified. Dawel, Salapingao and Gayaman were sampled in the morning and Nibaliw Central in the afternoon.

Water samples for microbial analysis were collected within 1 m from the surface of the water using sterile glass bottles which were kept inside an ice box until arrival at the laboratory. The MPN of total coliforms and *E. coli* were established according to procedures recommended by the American Public Health Association (APHA 1977). For convenience in the presentation of data, the dimensional unit, MPN/100 ml, is omitted.

During sampling, water temperature, salinity and turbidity were measured using a temperature-salinity bridge (Hamon Model 602) and turbidimeter (Toho Dentan Model FN-5), respectively. Cloudiness and tide level were also noted.

Results and Discussion

Dawel lies in a tributary, approximately 1 km from the mouth of Dagupan River. It is 3.4-m deep with sandy-muddy bottom. It receives pesticides from surrounding fishponds, garbages from homes and a nearby restaurant, wastes of domestic animals and human feces from about 70 inhabitants. The area produces an average of 86.6 t of oysters every year (Vidal, pers. comm.). Data from this study indicate that total coliform and *E. coli* MPN ranged from 170 to >24,000 and 2 to 16,000, respectively (Fig. 1).

Salapingao similarly lies in a tributary and is about 1 km away from the mouth of Dagupan River. It is 2.7 m in depth and the bottom is sandy-muddy. The area is residential, receiving garbage, animal and human wastes and oil slicks from passenger boats. Salapingao and Dawel are on opposite sides of Dagupan River. An average of 266.5 t of oysters are harvested from Salapingao per year (Abalos, pers. comm.). From available results, total coliform MPN ranged from 350 to >24,000 while *E. coli* MPN, from < 20 to 16,000 (Fig. 2).

Gayaman is further inland about 7 km from the mouth of Dagupan River. It is 3.8-m deep with sandy-muddy bottom. The area is lined with nipa and mangrove swamps on one side and rice paddies on the other. There is a single house along the river. Gayaman produces an average of 85 t of oysters every year (Gregoria, pers. comm.). Data show that total coliform and *E. coli* MPN ranged from 8 to 9,200 and < 2 to 5,400, respectively (Fig. 3).

Nibaliw Central is part of a small winding river and is about 1 km from the sea. It is 2-m deep with sandy-muddy bottom. The area is thickly populated, producing an average of 28 t of oysters every year (Quirimit, pers. comm.). Available data indicate that total coliform and *E. coli* MPN ranged from 110 to >24,000 and < 2 to >24,000, respectively (Fig. 4).

Hydrographic parameters such as salinity, temperature and turbidity were monitored to determine their influence on the coliform density. The roles of tidal level and incident sunlight were also considered.

The phenomenon of salinity exerting a bactericidal effect on fecal bacteria was revealed by Wood (1976) and is corrobrated in this study. The lowest total coliform and *E. coli* counts were observed during high salinities (28-31 ppt) in all study areas. Some lowest counts were noted when salinities were low (3.8 ppt in Salapingao; 12.6 ppt in Nibaliw Central). Under these circumstances, turbidity of the water was rather high (21 ppm in Salapingao; 11 ppm in Nibaliw Central). Turbidity which is a measure of the amount of suspended particles in the water column may also influence bacterial density. Highly turbid waters may contain large amounts of phytoplankton, the metabolic products of which can exert a bactericidal effect on fecal bacteria (Wood 1976). Furthermore, fecal bacteria are known to adhere to suspended particles and thereby their population is reduced (Wood 1976). This probably explains the low total coliform and *E. coli* counts observed from water samples in spite of low water salinity.

The highest total coliform and *E. coli* counts were noted at low salinities (1.5-12.3 ppt) during the wet months (July-October) when the rivers were diluted with freshwater runoff.

High water temperature and incident sunlight can kill coliforms and *E. coli* (Wood 1976). Also, bacterial levels in the water vary with changes in the tide (Vasconcelos et al. 1969). Figs. 1-4 indicate that these parameters have no valid significance to the total coliform and *E. coli* populations.

On the whole, Dawel, Salapingao, Gayaman and Nibaliw Central are, by foreign standards, prohibited and unfavorable areas for oyster growing because the coliform and *E. coli* MPNs generally far exceeded the microbiological limits set by the United States Public Health Service, Italy and France for approved and satisfactory oyster-growing areas. This study underscores the need to depurate the oysters harvested from these areas prior to raw consumption, and to identify clean, although non-traditional, sites for oyster-growing, if oysters are to be pursued as export products.

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The authors thank Ms. Grace De Vera-Fontanilla of the Philippine Human Resources Development Center, Mr. Benjamin D. Fontanilla of the Seafarming Research and Development Center, Mr. Hideo Takei, Mr. Takumi Takeuchi and Mr. Hiroshi Ayabe of the Japan International Cooperation Agency for the unwavering support throughout this study.

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*Seafarming Research and Development Center Contribution No. 2

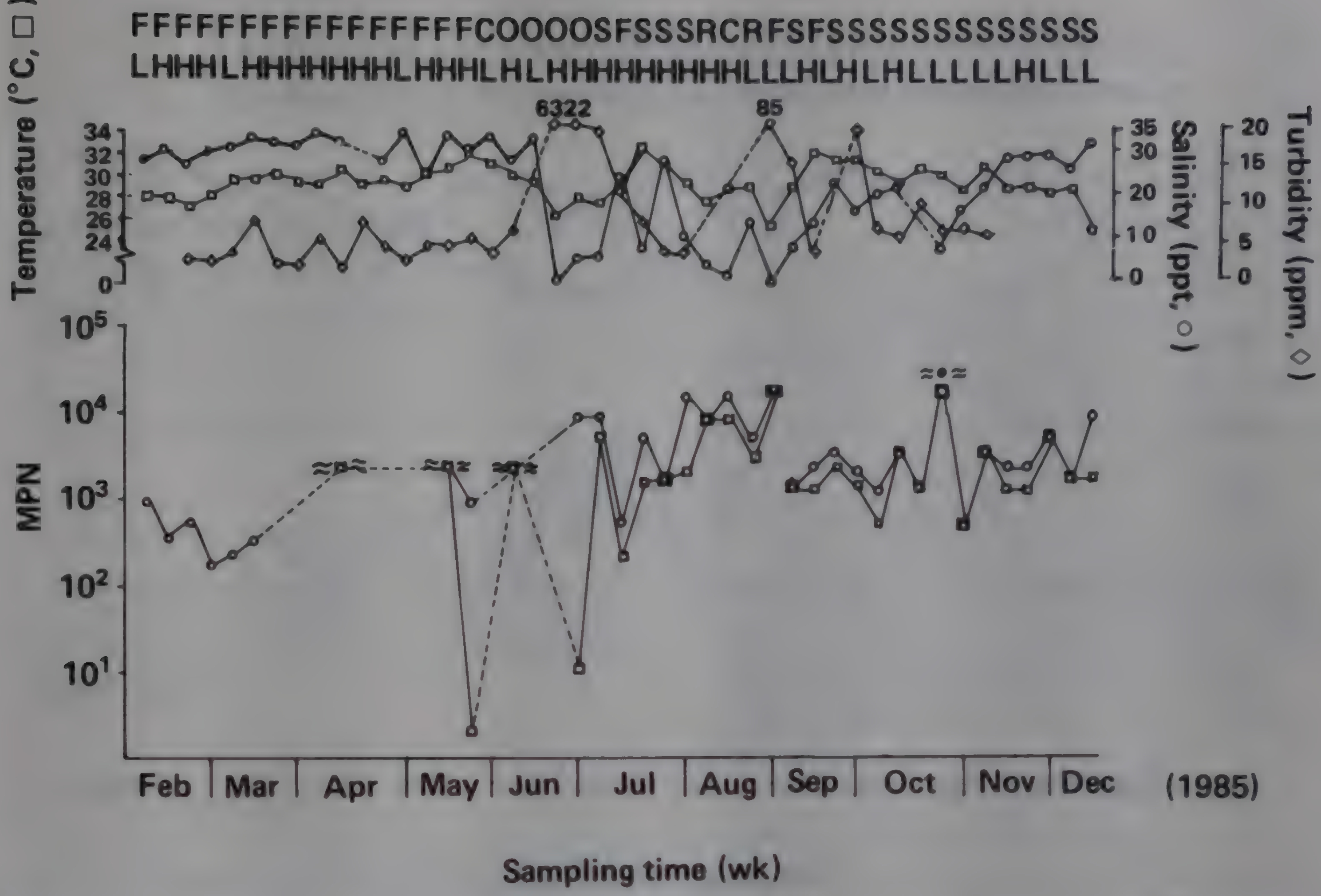


Fig. 1. Total coliform (○) and *E. coli* (□) MPN of water samples from Dawel. S = sunny; F = fair; C = cloudy; O = overcast; R = rainy; H = high tide; L = low tide; ↓ = less than; ≈ = greater than or equal to.

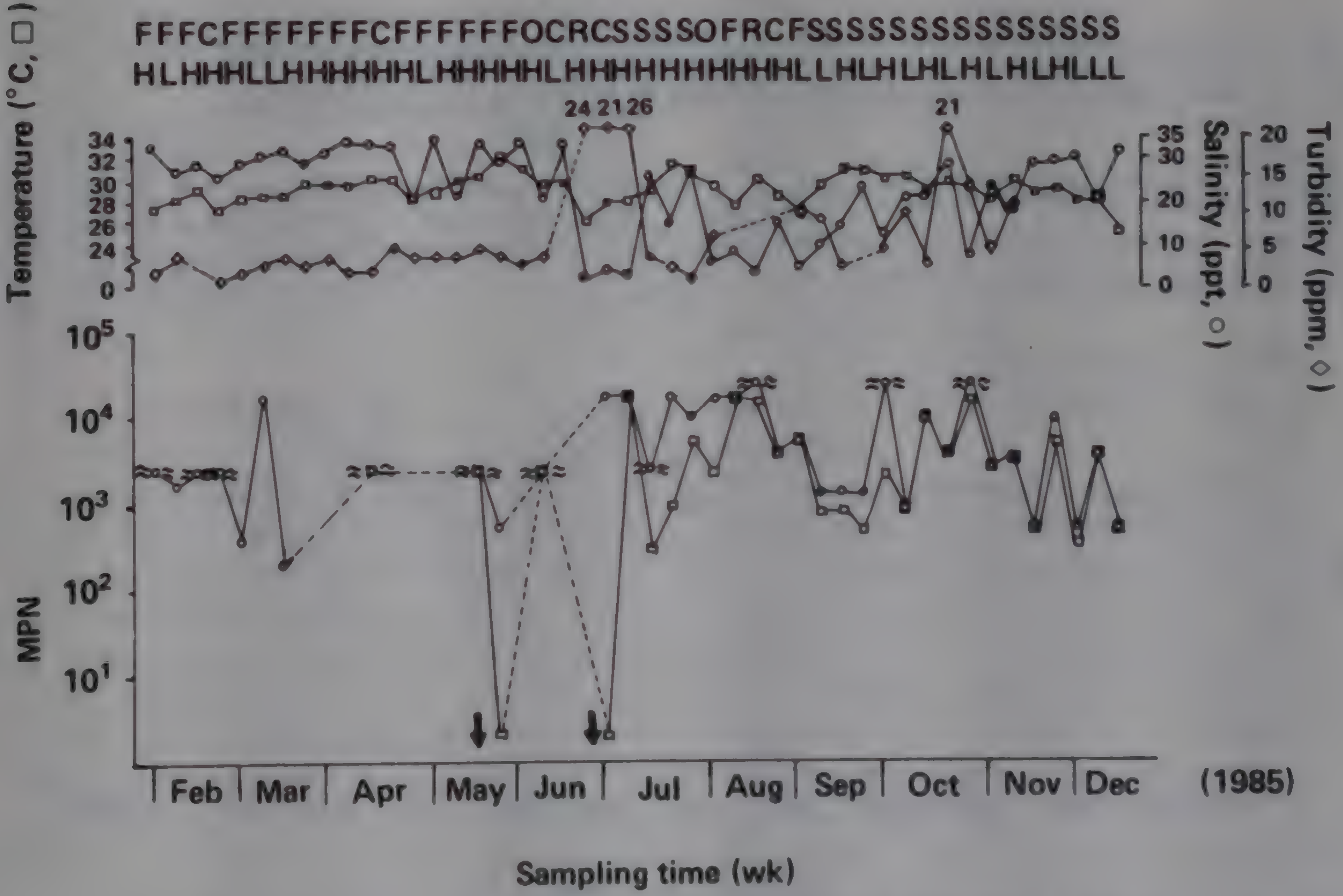
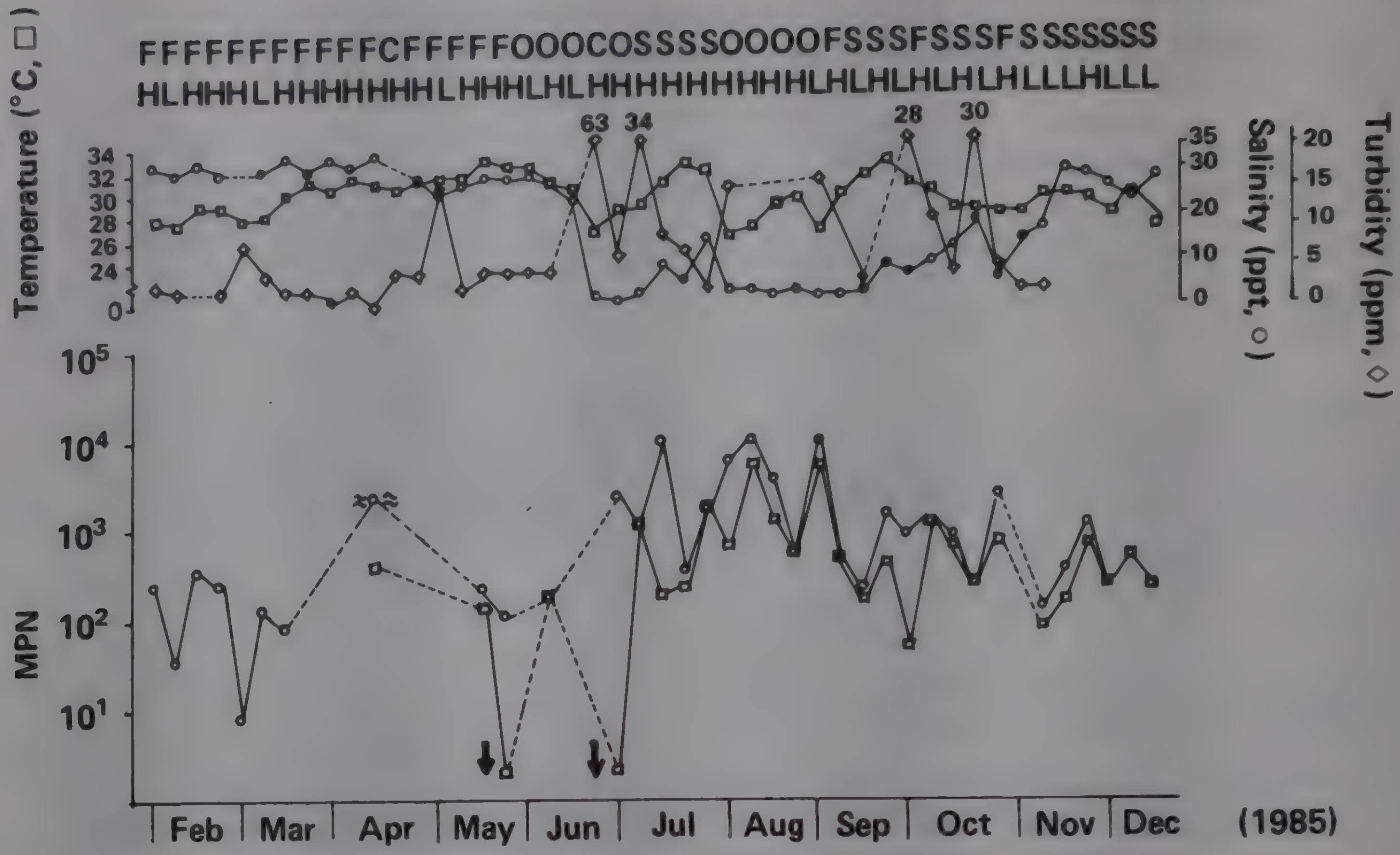
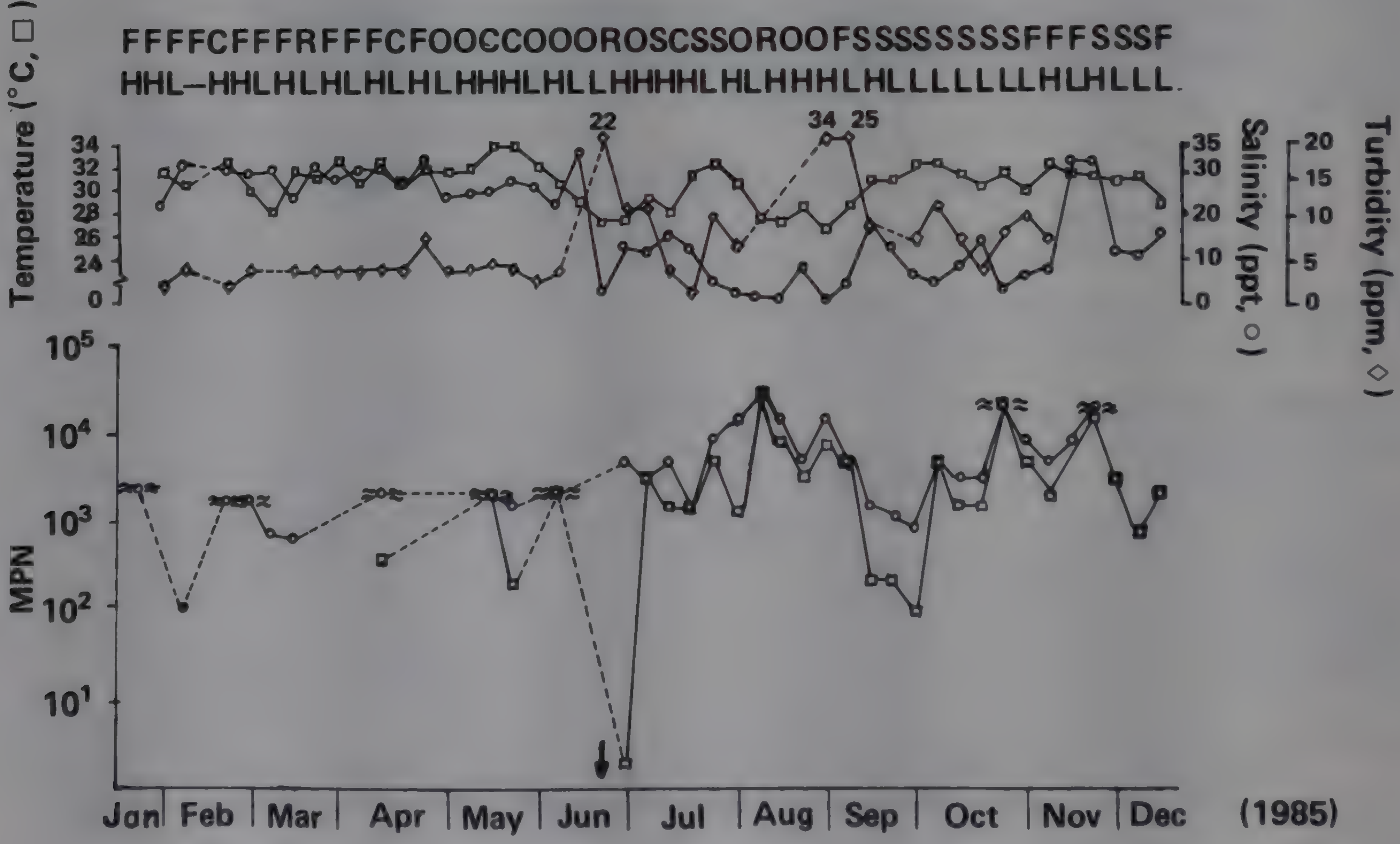


Fig. 2. Total coliform (○) and *E. coli* (□) MPN of water samples from Salapingao. S = sunny; F = fair; C = cloudy; O = overcast; R = rainy; H = high tide; L = low tide; ↓ = less than; ≈ = greater than or equal to.



Sampling time (wk)

Fig. 3. Total coliform (○) and *E. coli* (□) MPN of water samples from Gayaman. S = sunny; F = fair; C = cloudy; O = overcast; R = rainy; H = high tide; L = low tide; ↓ = less than; ≈ = greater than or equal to.



Sampling time (wk)

Fig. 4. Total coliform (○) and *E. coli* (□) MPN of water samples from Nibaliw Central. S = sunny; F = fair; C = cloudy; O = overcast; R = rainy; H = high tide; L = low tide; ↓ = less than; ≈ = greater than or equal to.

Household Demand for Fish in Iloilo City, Philippines

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POSADAS, B.C. 1986. Household demand for fish in Iloilo City, Philippines, p. 451-453. *In* J.L. Maclean, L.B. Dizon and L.V. Hosillos (eds.) The First Asian Fisheries Forum. Asian Fisheries Society, Manila, Philippines.

Abstract

A survey of 336 households in Iloilo City, Philippines, produced the following patterns of fish consumption behavior: Households are not very sensitive to increases in the price of various fish species; households purchase less milkfish when the price of other fish species rises; the demand for other fish species falls when the price of milkfish rises; the demand for fish decreases as the prices of beef and chicken increase; the price of pork does not significantly influence the demand for fish; when their incomes increase, households increase purchases of fish; big households do not necessarily consume more fish than small households, except for milkfish; households with higher formal educational background consume more fish than beef and pork; male and female household members consume similar quantities of fish; and households with better access to fish buy relatively more fish, except milkfish. The policy implications of these findings are discussed.

Introduction

This study seeks to understand Ilonggo households as consumers of fish as a paradigm for Philippine households. Families which depend on fish as primary protein source maximize satisfaction from their consumption of certain quantities and species of fish under certain conditions. Understanding such behavior will enable government and private enterprise to formulate policies according to their respective objectives.

Specifically, this study aims to: (1) identify the factors affecting the demand for fish by households in Iloilo City; (2) analyze the fluctuations of household demand for fish in this city; and (3) derive data that can be bases of policy implications appropriate to the fishing industry.

Methodology

The behavior of households in Iloilo City as consumers of fish was studied from 18 February to 18 March 1983. A cross-section of 336 households in 173 barangays of the six districts of Iloilo City was surveyed. The methodology used here has a socioeconomic conceptual framework.

The household demand for fish was considered determined by the following factors (Bell 1958; Nash 1969; Callaghan 1977; Arief 1980; Coslit et al. 1980; Posadas 1985; and Posadas et al. 1985):

- Normally, households tend to buy more fish when it is cheap.
- Households tend to buy more fish if beef, chicken, or pork is relatively more expensive.
- When households earn more income, they seem to buy more fish. They also shift to better or other commodities.
- The quantity of fish consumed tends to be higher in households with more adults.
- The average education of husband and/or wife determines the extent by which the household can accept new species or new ways of preparing fish. The more tolerant heads of families are to changes, the more households tend to consume fish because of its inherent low calories and fat content and high-quality protein.
- Males and females consume approximately the same quantity of fish.
- The accessibility of fish in terms of distance, fare and purchasing convenience encourages households to buy more fish.
- Household desire and preferences for fish lead to consumption of more fish than meat.

In estimating household demand for fish, the significant ratio found was the constant elasticity of demand (CED) or the double-logarithmic transformation. The estimated coefficients measure the sensitivity of household fish demand to a change in any of the determinants. For example, the absolute value of the estimated coefficient for the price of fish may be less or greater than unity. If it turns out to be less than one, then households are not sensitive to a price increase.

If the estimated coefficient of the income of households is less than zero, households reduce fish consumption when their income rises; they tend to shift to other goods of better quality. When the coefficient is positive, they consider fish as a normal commodity. To be more specific, fish to them is a necessity if the income elasticity coefficient is positive but less than unity. Should it exceed unity, fish becomes a luxury. This information is vital to fisheries development planning. Only those fish species with positive income elasticities exhibit economic potential. Translated into a policy option, it means these fish species deserve more attention for further research and development.

To determine the sensitivity of household demand for fish to a change in any of the determinants, the concept of demand elasticity is generally used. The price elasticity is the sensitivity of fish demand to price changes. The cross-price elasticity of demand is the responsiveness of fish demand to a given change in the price of another commodity. The same procedure applies to the other determinants of household fish demand.

In this paper, three demand functions are presented: demand for all species; demand for milkfish; and demand for all other species, namely, yaito tuna, nemipterids, goatfish, anchovy, Spanish mackerel, sand whiting, small slipmouth, shrimp fry, yellowfin tuna and others.

Results and Discussion

The results (Table 1) indicate that daily income positively determines the quantity of all species, milkfish and other fish species bought by households. All the fish species are considered normal goods with an income elasticity of demand of 0.42. The income elasticity of demand for milkfish was estimated at 0.25. An income elasticity of 0.37 was estimated for the other fish species. In short, milkfish and other fish species are considered necessities by the households interviewed.

The price of all fish species is a negative determinant of household demand for fish. The coefficient of price elasticity of demand for all fish species is -0.28, which indicates that households were not very sensitive to increases in the price of all fish species. The coefficient of price elasticity of the demand for milkfish is -0.73, which implies that the demand for milkfish is not strongly affected by its price. The price of other fish species showed insignificant influence over their quantity demanded by households.

The coefficients of cross elasticity of demand of all fish species and other fish species in relation to beef are -0.95 and -0.90, respectively. These indicate that the demand for all fish species and other fish species were influenced by the price of beef. This means that when beef became more expensive, households also tended to reduce fish purchases. Presumably, this phenomenon was due to the negative income effects of a general price increase which reduced purchasing power for the purchase of fish.

In relation to the price of chicken, households tended to consume less fish when chicken became more expensive by as much as 125% and 109% than all fish species and other fish species, respectively. The demand for milkfish was not significantly affected by the price of chicken.

Household demand for fish did not manifest significant responses to price fluctuations of pork.

Except for milkfish, household size did not show an important influence over household fish demand. Milkfish consumption increased by as much as 26% in response to doubling household size.

Education played a vital role in purchases of fish except for other fish species. When all members of the household have formal education, demand for all fish species and milkfish was 35% and 36% higher, respectively. The same amount of fish was consumed by both male and female members of the household.

The household demand for all fish species and other fish species would respond to a 100% improvement in their accessibility by as much as 9% and 14%, respectively. The demand for milkfish, however, seemed unaffected by any improvement in its accessibility.

The price of milkfish showed strong influence over the household's purchases of other fish species. When milkfish price doubled demand for other fish species dropped by 15%. In like manner, the price of other fish species played a significant role in purchases of milkfish. Households decreased milkfish consumption by 8% when other fish species became more expensive. These results imply that milkfish and other fish species were considered by the households to be complementary sources of fish protein.

With an average of all fish species purchased per week estimated at 1.38 kg and an average of 6.07 purchases/week, a household bought 8.29 kg of all fish species/week. Computed from the 1980 census, the 42,640 households bought a total of 353.65 t of all fish species/week. On a per capita basis, the average weekly fish consumption was 1.44 kg. Approximately 50.36 t of various fish species, therefore, were consumed by the residents of Iloilo City per day.

By the same analysis, each household bought 3.13 kg of milkfish/week or 0.54 kg/household member. On a daily basis, this amounts to 18.88 t of milkfish purchased by households. This estimate was around 32% of the average daily production of Iloilo fishponds in 1979.

Each household purchased 5.17 kg of other fish spcies/week. On a per capita basis, the weekly consumption of other fish species was 0.90 kg. A total of 31.48 t of other fish species were bought in the city every day by households. This figure is less than 25% of the average daily landing of commercial fisheries in Iloilo City in 1979 and 1980.

These findings can be the bases of policy formulation by government and private enterprise, but periodic studies should be made to update economic data and sociological trends because of changes in production, marketing and population.

With a relatively inelastic demand for fish, an increase in production of milkfish and landing of other species could lead to a substantial reduction in their selling prices. This could be advantageous to fish consumers but disadvantageous to fish producers. This situation requires further government support for the industry.

To improve the diets of households, those fish species with higher elasticities should be selected for further development. Milkfish, a cultured species, has both the economic potential and a higher consumer preference. Efforts should be taken to further develop milkfish and other species with desirable biological qualities and economic potentials.

To raise household purchases of fish, better distribution outlets and expanded storage facilities would make fish more accessible to households.

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Table 1. Regression results with quantity of household fish consumption as dependent variable.

Independent variable	All fish species	Milkfish	Other fish species
Intercept	25.03	0.56	39.25
Price of all fish species	–0.28*	–	–
Price of milkfish	–	–0.73**	–0.15**
Price of other fish species	–	–0.08*	–0.19ns
Price of beef	–0.95*	0.72ns	–0.90*
Price of chicken	–1.25**	–0.26ns	–1.09*
Price of pork	0.05ns	1.37ns	0.20ns
Household income	0.42**	0.25**	0.37**
Household size	0.16ns	0.26*	0.08ns
Education	0.35**	0.36**	0.20ns
Sex ratio	——	0.02ns	–0.11ns
Accessibility of fish	0.09**	0.04ns	0.14**
Desire and preference	0.16ns	–0.28ns	–0.01ns
R-squared	0.45	0.33	0.31
Standard error	0.51	0.44	0.59
F-ratio	26.47**	8.59**	11.11**
Number of households	331	206	284

* = Significant at 5%.
** = Significant at 1%.
– = Not applicable.
—— = Negligible value.
ns = Not significant at 1 or 5%.

Postharvest Spoilage of Shrimp *(Penaeus monodon)*

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REILLY, A., E. DANGLA and A. DELA CRUZ. 1986. Postharvest spoilage of shrimp *(Penaeus monodon)*, p. 455-458. *In* J.L. Maclean, L.B. Dizon and L.V. Hosillos (eds.) The First Asian Fisheries Forum. Asian Fisheries Society Manila, Philippines.

Abstract

Quality losses occur during postharvest handling of pond-cultured shrimp. This paper describes the chemical, sensory and microbiological changes which occur when shrimp are iced insufficiently and improperly and stored at elevated temperatures. As a general rule, for every 1°C above zero at which shrimp are stored, storage life at zero is reduced by one day. Recommendations for the elimination of quality losses during primary handling, with a view of meeting standards for export markets, are included.

Introduction

Shrimp are amongst the most valuable export products of the Philippines and the potential for increased production from pond culture is considerable. Over the past few years, there has been a gradual change from extensive to semi-intensive systems with harvests of up to 8 t/ha/year. With the increase in the volume of production arises the need to improve primary on-site handling of shrimp. The market for premium quality, head-on shrimp is currently undersupplied and such products can be obtained with immediate washing and icing upon harvesting and freezing with minimum delay.

Most of the brackishwater ponds in the Philippines are situated close to coastal areas where roads are poor or nonexistent and electricity supply erratic. As a result, insulated containers and ice have to be handcarried to harvesting sites and the produce similarly transported to the nearest road. Shrimp are frequently not iced immediately or insufficiently iced. In a previous report the effects of delay in icing on the quality of brackishwater shrimp were determined (Reilly et al. 1984a). This study simulated commercial chilling methods where shrimp were stored at temperatures above 0°C prior to freezing to compare spoilage characteristics of *Penaeus monodon* during storage in ice/water slurries at 0, 5 and 10°C.

Materials and Methods

Two storage trials were carried out on brackishwater shrimp from ponds close to Manila Bay. Samples of 15-20 shrimp/kg were harvested as previously described (Reilly et al. 1985) and ice-killed. These were immediately divided into three lots and transferred to ice/water slurries at 0, 5 and 10°C, kept in insulated boxes and transported to the laboratory of the University of the Philippines in the Visayas, College of Fisheries, where analyses were carried out within eight hours after harvest. Throughout the storage period the insulated boxes were kept at room temperature where ice was added twice daily and mixed to achieve uniform temperatures. Eight to ten shrimp were taken for sensory, chemical and microbiological analysis on each sampling day as previously described (Reilly et al. 1985).

Results and Discussion

The storage life of shrimp was based on cooked flavor scores assessed by five trained taste panelists. Linear regression analyses for the different storage temperatures are presented in Fig. 1. The storage life, based on a sensory score of 4, was 16, 11.5 and 6.5 days at 0, 5, 10°C, respectively, which approximated a loss of one day for every degree above 0°C at which shrimp were stored. At all temperatures, samples were rejected because of soft texture, ammoniacal odor and a bitter to strong sulphide taste. At 0°C heads remained firmly attached for two days, after which shrimp lost their value as prime quality head-on produce. Melanosis was delayed up to seven days by iced water at 0°C, because of the low concentration of oxygen in the chilling medium. When shrimp were stored in ice, melanosis occurred more rapidly (Reilly et al. 1984) than in chilled water at 0°C.

The results of the standard plate counts (SPC) are presented in Fig. 2. There was a significant decrease in the initial SPC when shrimp were stored at 0°C due to suppression of mesophilic growth which did not occur at the other two storage temperatures. Irrespective of the storage temperature, shrimp were considered spoiled when the SPCs at 20°C increased to 10^7 Colony Forming Units (cfu)/g and the hydrogen sulphide producers to 10^5-10^6 cfu/g. The latter group comprised 4.7, 3.7 and 2.2% of the SPC at 0, 5 and 10°C, respectively, when shrimp were spoiled, which are considerably lower values than those of a similar study on pelagic fish (Barile et al. 1985). Contamination of the chilling medium with gut contents was the reason for the high percentage of H_2S produced during storage of pelagic fish, which did not happen during storage of shrimp.

The changes in bacterial flora during storage are presented in Fig. 3. The initial flora were mesophilic and dominated by Enterobacteriaceae (20%), *Aeromonas hydrophila* (20%) and *Achromobacter* sp. (20%) and to a lesser extent by *Acinetobacter* sp. (13.3%), *Pseudomonas* sp. (13.3%), *Chromobacterium* sp. (6.7%) and *Vibrio cholerae* (67%). All the Enterobacteriaceae were identified as species of the genus *Enterobacter*. It is not unusual to find such high numbers of Enterobacteriaceae as animal manure is commonly used to fertilize ponds. This contrasts with the initial microflora of marine shrimp where Enterobacteriaceae are rarely isolated as part of the initial flora.

A. hydrophila forms part of the natural flora of brackishwater and under certain environmental conditions is a fish pathogen. It has also been reported as a human pathogen causing "travellers diarrhea" (Kipperman et al. 1984). The presence of *V. cholerae* as part of the natural flora is a cause for concern. The main reason for rejection of shrimp from Southeast Asia by Japanese health authorities is the presence of this organism. Table 1 shows the quantity of rejected shipments between 1980 1985 by Japan (Kitamur, pers. comm.). The figure for the Philippines amounts to about 0.1% of total export of shrimp. At all storage temperatures *A. hydrophila* was the principal spoilage organism. Similar results were obtained with storage of pelagic fish in chilled seawater (Barile et al. 1985). During ice storage trials with *Penaeus monodon* (Reilly et al. 1985), *Alteromonas putrefaciens* and *Pseudomonas* sp. were the dominant spoilers.

The changes in percentage of K-value during storage at different temperatures are shown in Fig. 4. Very fresh shrimp had a value of 2.03% which steadily increased to 43.5, 50.6 and 35% at 0, 5 and 10°C, respectively, when samples were rejected. These values are very much lower than those reported for mackerel (Barile et al. 1985) or tilapia (Saluan-Abduhasan 1985). Similar values were obtained when brackishwater prawns were stored in ice (Reilly et al. 1985). The rate of freshness loss as expressed by K-values was twice as fast at 10°C than at 0°C.

Values for total volatile nitrogen (TVN) during storage are shown in Fig. 5. At rejection the TVN values were 19, 21.4 and 20 mg/100 g at 0, 5 and 10°C, respectively, although values fluctuated during storage.

The increase in TVN levels was due to bacterial metabolism and shrimp had spoiled before they reached rejection limit set by Japanese and Australian marketing systems of 30 mg/100 g (Montgomery et al. 1970). The low TVN values are most likely due to the leaching effect of the ice water.

Recommendations

This study shows the presence of pathogenic bacteria as part of the natural microflora of pond-reared shrimp. As these organisms are mesophiles, storage at elevated temperatures can permit their survival and growth. The most effective method of removing the bacteria is washing in clean running water. Most of the bacteria contaminating freshly-harvested shrimp are surface contaminants which are readily removed by effective washing. It is recommended that shrimp are washed with clean iced water immediately after capture. If possible chlorine should be added to a concentration of about 20-50 mg/l. This method will have the dual effect of removing in excess of 90% of surface bacteria and instantly killing the shrimp to preserve their fresh quality. Special care should be given to shrimp which have been handpicked from the bottom of ponds after draining as experience shows that these are heavily covered with mud.

If shrimp are to be sorted and graded at the pond site, portable sorting tables are required, and the temperature must be maintained below 5-10°C. The common practice of dumping shrimp onto the muddy banks of ponds for sorting only leads to further contamination of produce.

Shrimp should be immediately packed in ice or ice-water at 0°C and transported with minimum delay to the processing plant. Harvests should be scheduled to allow processing and freezing within 24 hours after harvesting.

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Table 1. Samples of raw frozen shrimp rejected by Japan, 1980-1985, due to contamination by *Vibrio cholera.*

Country	Quantity/t
Philippines	36.43
Thailand	58.6
Indonesia	28.8
India	58.14
Taiwan	23.97

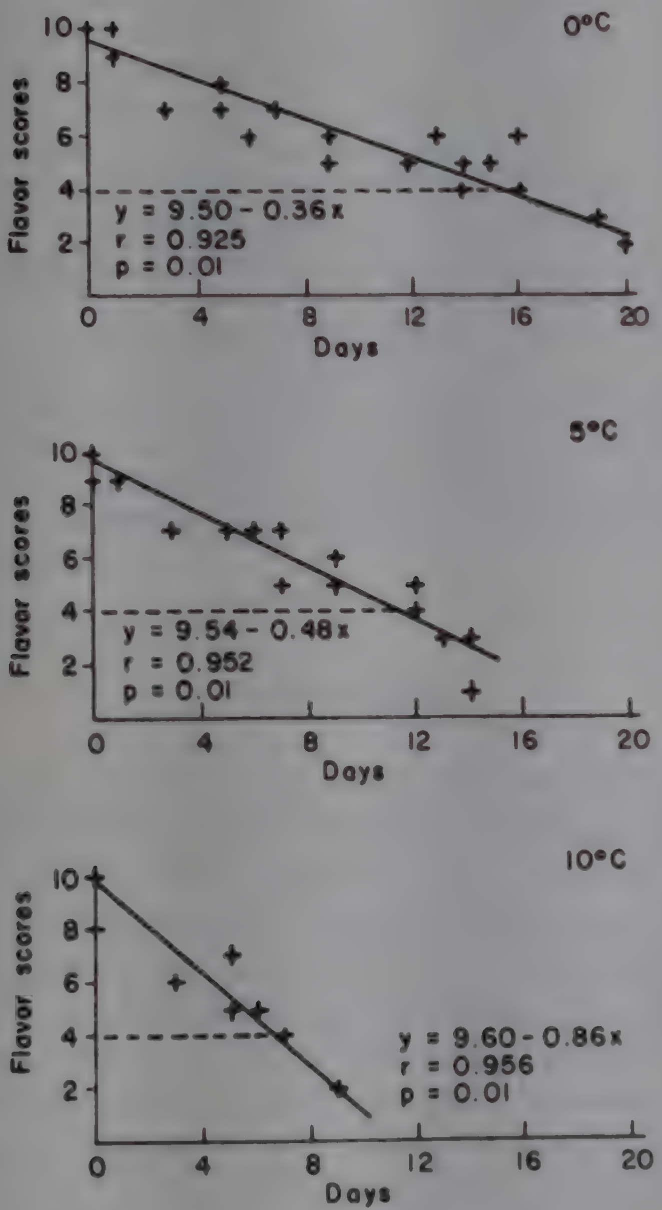

Fig. 1. Correlation between cooked flavor scores of shrimp at different storage temperatures and storage time.

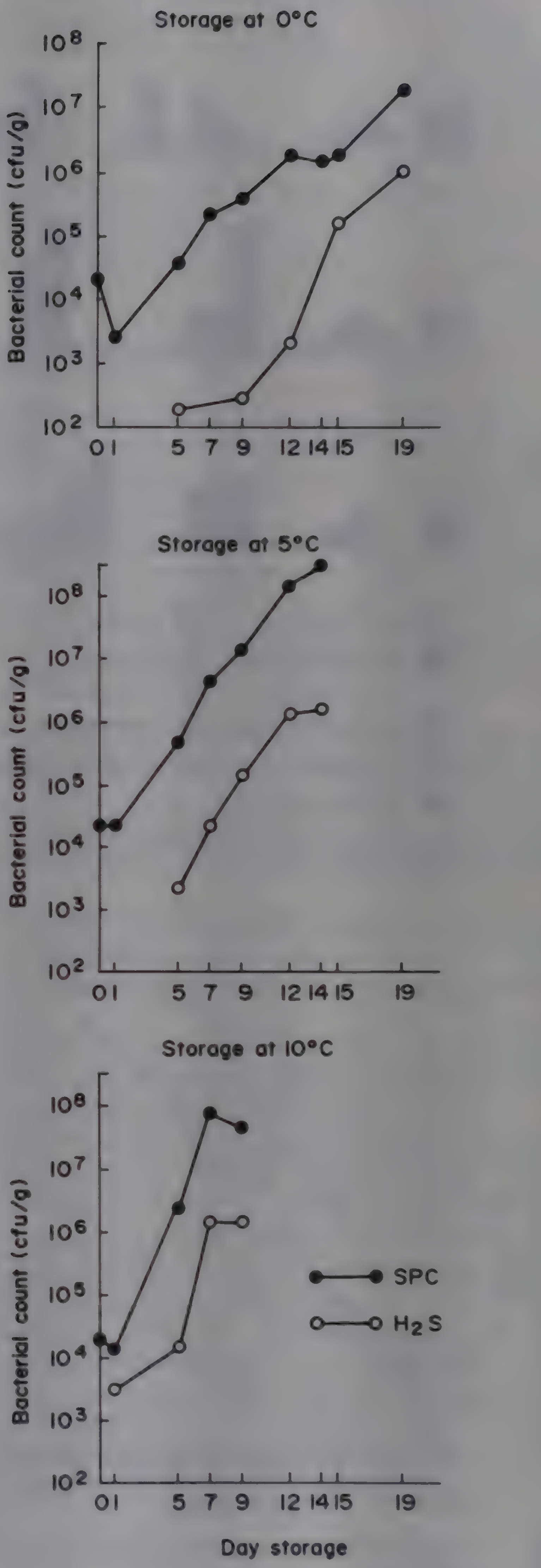

Fig. 2. Changes in the Standard Plate Count (SPC) and hydrogen sulphide producers (H_2S) count at 20°C during shrimp storage at different temperatures.

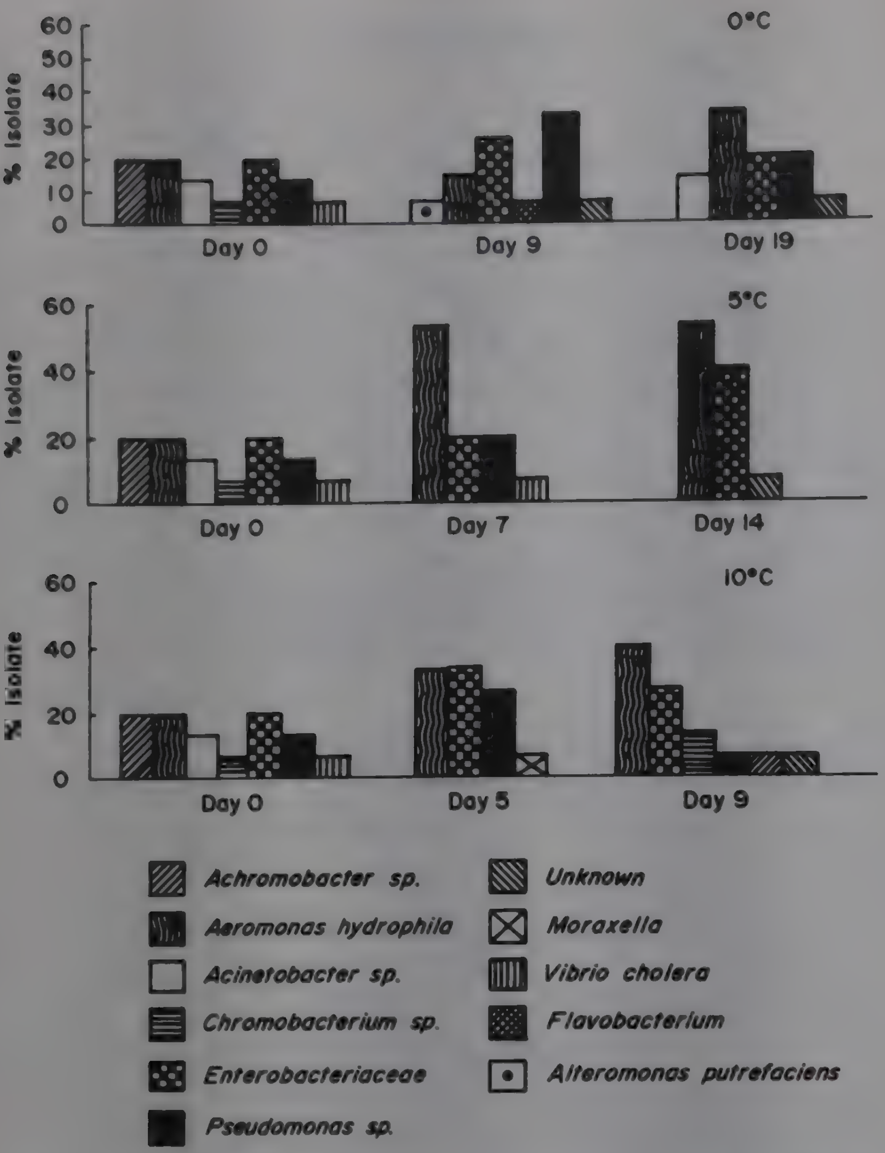

Fig. 3. Changes in the bacterial flora of shrimp during storage at different temperatures.

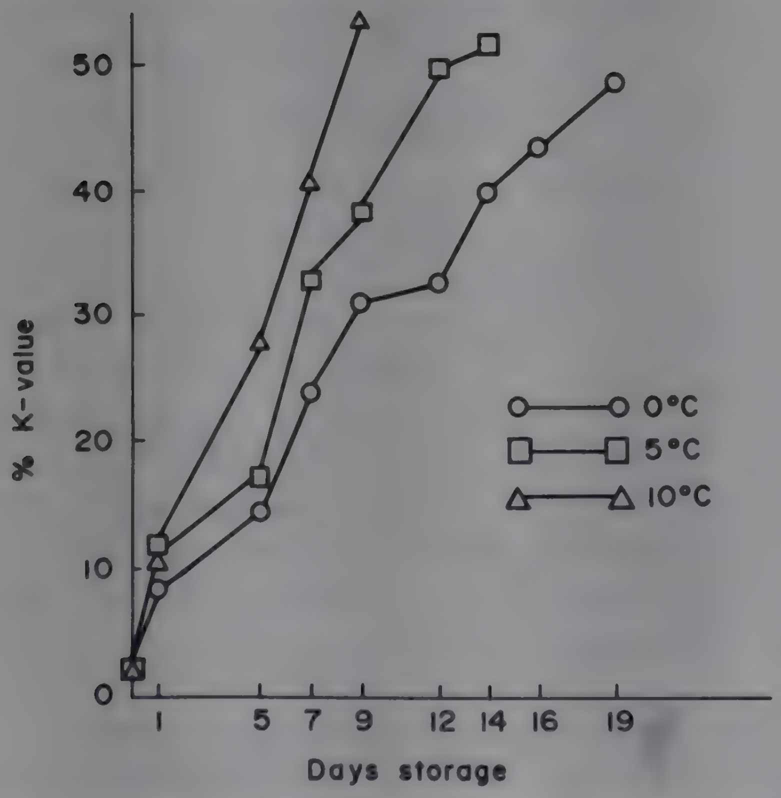

Fig. 4. Increase in %K-value with shrimp storage time at different storage temperatures.

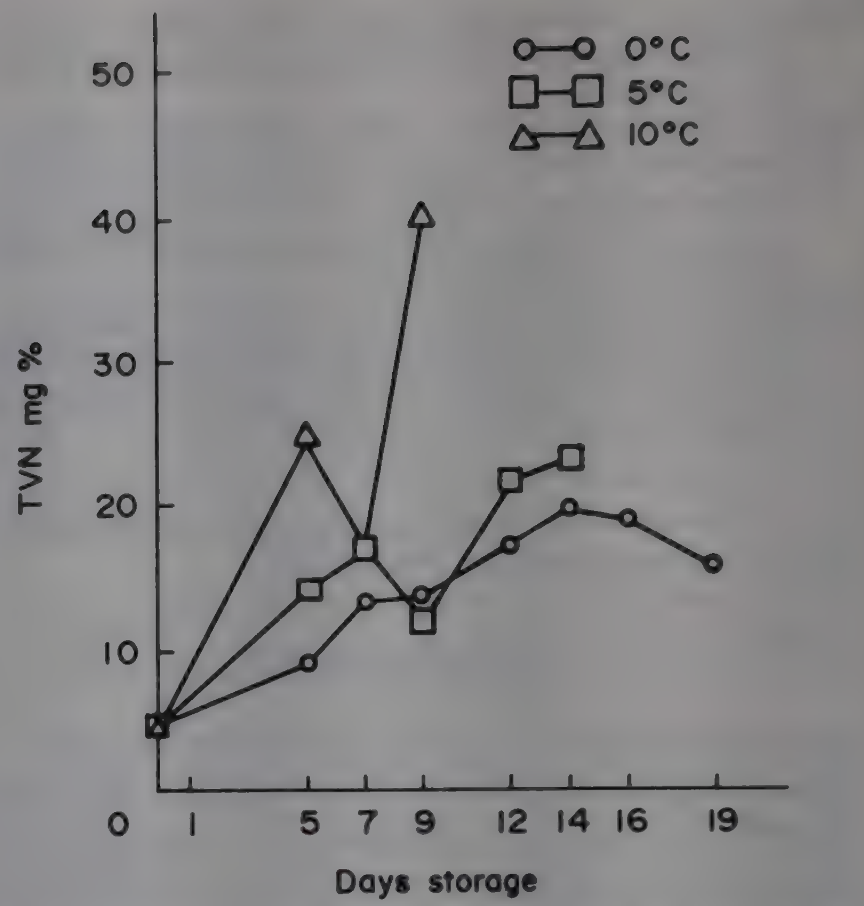

Fig. 5. Changes in Total Volatile Nitrogen (mg %) with storage time at different temperatures.

Quality Loss and Wastage in the Processing and Preparation of Fresh Fish in Sri Lanka

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SUBASINGHE, S. and J.C. GODAKANDA. 1986. Quality loss and wastage in the processing and preparation of fresh fish in Sri Lanka, p. 459-462. *In* J.L. Maclean, L.B. Dizon and L.V. Hosillos (eds.) The First Asian Fisheries Forum. Asian Fisheries Society, Manila, Philippines.

Abstract

Nearly 85% of the fish landed in Sri Lanka are consumed fresh. The relationship between the total quantity of fish landed and the actual amount of fish protein available to the population depends on a number of factors such as the composition of the catch, methods of processing and preparation, and eating habits of the population. *Katsuwonus pelamis, Thunnus albacares, Lutjanus malabaricus, Leiognathus* sp., *Amblygaster sirm, Anchoviella indica, Scoliodon palasorrah, Oreochromis* sp. and *Penaeus monodon* were studied for their dressing losses, cooking losses, table waste and nutrient availability. The highest total protein availability was observed in *Thunnus albacares* (149.7 g/kg) while *Leiognathus* sp. showed the lowest (83.1 g/kg). *Amblygaster sirm* recorded the highest fat content of 35.7 g/kg. *A. sirm* and *Anchoviella indica* showed a very high level of available calcium (296 and 357 mg/kg, respectively). This study also revealed the inadequacy of the fish protein intake of the population, the protein intake from fish amounting to only 70% of the minimum recommended intake of fish protein, calculated on the basis of FAO recommended protein intake. The flesh recovery rate was found to be approximately 55% of the total quantity of the fish landed.

Introduction

In the developing countries of South and Southeast Asia fish forms an important source of animal protein in the diet (Guha 1962). However, data available on fish consumption are based on statistics of production, exports and imports and are of limited value in assessing the role of fish in the nutrition of the population. The relationship between total fish landed and actual amount of fish protein, as well as other nutrients available to the population, depends on a number of factors including the composition of the catch, methods of processing and preparation, and eating habits of the population.

This study examines the physical losses and wastage during processing and preparation of fresh fish marketed in Sri Lanka and attempts to quantify the intake of the major nutrients -- protein, fat and calcium -- by the population through the fish consumption.

Materials and Methods

Samples were collected from nine different species representing groups A-F (Table 1). Fish of uniform size were used, the number of fish sampled varying from 3 to 15 depending on body size.

The length, total weight and weight of the major body constituents (head, viscera and fins) of individual fishes were recorded. Samples of fish flesh were collected from the dressed fish and homogenized and subsamples were analyzed in duplicate for moisture, protein, fat and total ash.

The dressed fish were cooked for 15 min. in a known volume of water sufficient to cover the fish completely. In keeping with the culinary practices in Sri Lanka, the large food fishes representing groups A, B, D and E of Table 1 were diced into pieces of less than 4-cm thickness prior to cooking.

The volume of cooking water was noted and samples analyzed for protein. The weight of the edible and nonedible constituents of the cooked fish were recorded separately.

Samples of cooked fish flesh were collected and homogenized and subsamples analyzed in duplicates for moisture, protein, fat, total ash and calcium.

Moisture content of the raw/cooked fish flesh was determined by drying a known weight of flesh to a constant weight in an oven at 105°C. To determine the total ash content, a known weight of the moisture-free sample (approximately 3-5 g) was charred and incinerated at 550°C for 20 hours in a muffle furnace (AOAC 1975). The sample was cooled in a desiccator and weighed to determine the total ash content. A portion of the ash was extracted with concentrated HCl and the extract used in the determination of calcium (AOAC 1980). Total nitrogen was determined by the Kjeldhal method. Fat was determined by the method of Bligh and Dyer (1959).

Results

Nearly 85% of the fish landed in Sri Lanka consist of marine species, mainly small pelagics (Table 1) which form nearly 40% of the total fish landed. The balance consists mainly of nearly twenty species of large food fish. The major freshwater species are tilapia (*Oreochromis* sp.) and carp (*Cyprinus* sp.). The species of fish landed have diverse body shapes and vary in chemical composition.

The major loss in large food fish takes place during dressing prior to storage, transportation or retailing. The small food fish are marketed as whole fish and are normally dressed prior to preparation for cooking. Dressing losses are generally low in small food fish with the exception of silverbelly (50%). Among the large food fish studied, shark showed the highest dressing loss of 35.9% (Table 2).

Protein and fat contents of cooked flesh were higher than that of raw flesh. This increase could be attributed to the lower moisture content of the cooked flesh (Tables 4 and 5). The recovery of cooked flesh was highest in sardine followed by yellowfin tuna and anchovy. Silverbelly and tilapia recorded lowest recovery of cooked flesh.

Table 6 shows the available nutrients in cooked flesh expressed as the quantity available in a kilogram of whole fish. Availability of protein was highest in yellowfin tuna. In general, blood fish species and small pelagic fish showed relatively high levels of protein availability with the exception of silverbelly which had the lowest recovery. The slightly higher level of protein observed in shark flesh, compared to other species studied, could be attributed to the presence of non-protein nitrogen in the flesh. The flesh of elasmobranchs has been shown to contain very high levels of non-protein nitrogenous compounds accounting for 34-38% of total nitrogen as compared to 9-18% in other varieties of fish (Stansby 1953; Geiger and Borgstrom 1962).

The amount of protein appearing in cooking water varied from 7.4 to 14.3 g/kg of whole fish in the species studied. The relatively high level of soluble protein observed in shark could again be attributed to the presence of non-protein nitrogen in cooking water. Hence, protein values for shark in Table 6 have been multiplied by a correction factor of 0.8 to allow for the non-protein nitrogen, in calculating the available protein in Table 7. The sardines had the highest level of available fat followed by the freshwater fish species tilapia.

The edible flesh content of the cooked fish varied widely and depended on the amount of table waste. When expressed as a percentage of the weight of the whole fish, tilapia showed the highest plate waste while the small food fish were generally shown to have a plate waste lower than that of large food fish with the exception of blood fish species (Table 3).

The small pelagic species were also rich in available calcium with anchovy and sardine recording high values. The availability of protein, fat and calcium of fish listed in Table 1, is given in Table 7. In estimating the nutrient availability in group B2, the values obtained for the recovery rate and the chemical composition of the cooked flesh of snapper (B1) was used. Similarly for group D2 fish the values obtained for shark (D1) were used. However, skates and rays had a recovery rate of 41.5% for raw fish flesh with skin (Kizevetter 1972). In this study the recovery of cooked flesh for group D2 species was assumed to be 32%, leaving an allowance of 9.5% for the weight of the skin and the weight loss during cooking.

The average of the values obtained for the percentage recovery of cooked fish flesh and the chemical composition of the cooked flesh of the various species were used in calculating the available nutrients in group G1 fish.

Discussion

The total fish supply of a country depends on the level of fish production, the quantity of fish exported and the quantity imported. Per capita availability of fish depends on total fish supply, population size and postharvest losses in the trade. Thus, to obtain a more accurate estimate of fish availability, allowance has to be made to cover both qualitative and quantitative losses of fish.

Loss in fish quality could be either due to chemical changes in fish or to microbiological activity. The most serious loss in nutrients occurs during sun-drying of fish and chemical changes which decrease the biological value of fish protein.

Over 85% of the fish landed in Sri Lanka are sold fresh; thus, fish availability is mainly influenced by the physical wastage during processing and preparation. Table 7 presents the average daily per capita consumption of protein, fat and calcium by the population through fish consumption.

The results in Table 7 can also be used to estimate the percentage recovery of fish flesh from fish landed. The total protein availability of fish landed in 1983 has been shown to be around 24,320 t and is equivalent to 121,600 t of fish flesh with 20% protein. Thus the recovery of fish flesh from the total quantity of fish landed in 1983 could be assumed to be around 55% of the total weight.

In this study the average daily per capita production of fish was estimated at 40.2 g (Table 7). The actual protein intake from this amount of fish has been estimted to be 4.4 g. The World Health Organization recommended minimum intake of protein for an adult is around 55 g/day. This value is based on a mixed dietary protein with a New Protein Utilization (NPU) value of 60 (FAO 1957; FAO 1973). Thus, assuming animal protein to provide 15% of the total protein requirement of the national diet, the total animal protein requirement will be around 8.0 g/person/day. In Sri Lanka, fish provides around 70% of the animal protein intake of the population and as such, based on the FAO-recommended protein intake, the minimum fish protein requirement of the nation is around 5.6 g/person/day. However, assuming a NPU value of 90 for protein in the cooked fish flesh, the fish protein intake of the population falls short of the minimum fish protein requirement by nearly 30%.

An examination of the results in Table 6 and 7 also points to the similarity in protein availability of the large food fish such as yellowfin tuna and skipjack and the small pelagic species such as sardine and anchovy in Sri Lanka. The small pelagic species and freshwater fish species such as tilapia often have a lower market value than large marine food fish and are less popular among consumers. The low market value of the small pelagic species has been attributed to a number of reasons such as their high perishability, the beliefs that they are less nutritious and that they contain less edible flesh. Shrimp, a relatively high-priced seafood, ranked very low in protein availability.

The small pelagic species and tilapia were also shown to be rich in calories due to their high fat content and thus could provide, in addition to protein, extra calories to the diet. This is very significant as far as the nutrition of the low-income groups is concerned where malnutrition is prevalent. The small pelagic species were also shown to be rich in calcium because of the presence of edible bones in the fish flesh.

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Table 1. Major species landed in Sri Lanka grouped by habitat and taxonomy.

Group		Species	Species represented	% landed in 1983	
(A) Blood fish; pelagic, medium-large, elliptical body	1	Skipjack *(Katsuwonus pelamis)*	Skipjack *(Katsuwonus pelamis)*	6.4	(A 1)
	2	Yellowfin tuna *(Thunnus albacares)* and other Thunnidae	Yellowfin tuna *(Thunnus albacares)*	9.2	(A 2)
(B) Large food fish, flattened body	1	Trevally (Carangidae)		3.9	(B 1)
	1	Other species of Mullidae, Lethrinidae, Serranidae, etc.	Red snapper *(Lutjanus malabaricus)*	10.7	(B 2)
(C) Small pelagic species, elliptical body	1	Silverbelly (Leiognathidae)	Silverbelly (*Leiognathus* sp.)	12.5	(C 1)
	2	Sardines (*Sardinella* sp.), trenched sardine *(Amblygaster sirm)*, mackerel (Scrombidae), anchovy (*Anchoviella* sp.) etc.	Trenched sardine *(Amblygaster sirm)* and Anchovy *(Anchoviella indica)*	26.8	(C 2)
(D) Elasmobranchs	1	Sharks (Carcharinidae)	Grey dog shark *(Scoliodon palasorrah)*	4.0	(D 1)
	2	Skates (Rajidae) and rays (Trygonidae)	Sting ray *(Himantura uarnak)*	4.7	(D 2)
(E) Freshwater fish species	1	Tilapia (*Oreochromis* sp.) and carp (*Cyprinus* sp.)	*Oreochromis niloticus*	16.3	(E 1)
(F) Shellfish	1	Prawns, crabs and lobsters	*Penaeus monodon*	3.0	(F 1)
(G) Miscellaneous species	1	Spanish mackerel *(Scomberomorus commerson)* and few other species	none	2.5	(G 1)

Table 2. Length, weight and % dressing loss of fish

Variety	No. samples	Mean length (cm)	Mean weight (g)	Dressing loss (g)	Dressing loss (% of total weight)
Skipjack tuna	8	47.0 ± .44	2,733.4 ± 378.0	699.3 ± 67.8	25.7
Yellowfin tuna	3	60.9 ± 2.49	2,650.0 ± 282.0	816.9 ± 70.1	30.9
Snapper	8	47.0 ± .59	2,126.3 ± 132.0	718.8 ± 39.0	33.7
Shark	8	69.6 ± 2.86	2,099.3 ± 397.0	755.4 ± 149.0	35.9
Tilapia	7	23.8 ± .47	486.9 ± 91.6	161.9 ± 27.0	33.4
Sardine	10	14.5 ± 1.00	45.5 ± 9.5	10.1 ± 02.38	22.1
Anchovy	18	6.7 ± 0.43	4.56 ± 0.84	0.75 ± 0.11	16.6
Silverbelly	10	8.5 ± 1.02	18.15 ± 6.31	9.09 ± 02.84	50.6
Shrimp	18	7.3 ± 1.0	4.9 ± 0.8	1.68 ± 0.20	34.3

Table 3. Table waste and recovery of edible fish

Variety	Mean weight (g)	Cooked weight of dressed fish (g)	Table waste % of whole fish (g)	Recovery of cooked edible flesh % of total weight
Skipjack tuna	2,733.0 ± 378.0	1,489.7 ± 242	134.8 ± 10.4 (5%)	49.5
Yellowfin tuna	2,650.0 ± 282.0	1,643.0 ± 184	159.6 ± 23.0 (6%)	56.9
Snapper	2,126.3 ± 132.0	1,177.5 ± 94	170.9 ± 22.4 (8%)	47.3
Shark	2,099.3 ± 397.0	1,157.0 ± 234	206.5 ± 37.0 (10%)	45.3
Tilapia	486.9 ± 92.0	265.7 ± 51.2	68.4 ± 13.9 (14%)	40.5
Sardine	45.5 ± 9.5	28.4 ± 1.9	2.19 ± 0.3 (5%)	57.9
Anchovy	4.56 ± 0.84	2.78 ± 0.72	0.32 ± 0.05 (7%)	53.9
Silverbelly	18.15 ± 6.3	7.79 ± 2.6	1.06 ± 0.28 (6%)	37.0
Shrimp	4.9 ± 0.8	2.3 ± 0.6	0.59 ± 0.07 (12%)	34.9

Table 4. Proximate chemical composition of raw fish flesh.

Variety	Moisture % t/w	Ash % t/w	Protein % t/w	Fat % t/w
Skipjack tuna	74.2 ± 0.13	1.5 ± 0.02	21.8 ± 0.29	1.8 ± 0.04
Yellowfin tuna	72.0 ± 0.85	1.6 ± 0.04	22.9 ± 0.18	2.9 ± 0.11
Snapper	77.1 ± 0.19	1.8 ± 0.05	17.1 ± 0.14	3.2 ± 0.07
Shark	72.5 ± 0.03	1.2 ± 0.03	23.4 ± 0.03	1.2 ± 0.02
Tilapia	74.7 ± 0.14	1.5 ± 0.06	19.2 ± 0.23	3.5 ± 0.05
Sardine	71.9 ± 0.01	2.1 ± 0.13	19.9 ± 0.25	4.3 ± 0.07
Anchovy	77.5 ± 0.19	1.6 ± 0.05	17.6 ± 0.11	1.8 ± 0.16
Silverbelly	78.5 ± 0.40	1.4 ± 0.04	18.0 ± 0.14	1.2 ± 0.04
Shrimp	78.1 ± 0.4	1.1 ± 0.07	16.5 ± 0.14	2.8 ± 0.11

Table 5. Proximate chemical composition of cooked fish flesh.

Variety	Moisture % t/w	Protein % t/w	Fat % t/w	Calcium mg/100 g
Skipjack tuna	70.6 ± 0.49	24.5 ± 0.26	2.3 ± 0.05	16.1 ± 1.16
Yellowfin tuna	68.0 ± 0.09	24.9 ± 0.11	3.3 ± 0.15	not available
Snapper	72.9 ± 0.16	20.8 ± 0.31	3.9 ± 0.12	29.0 ± 2.85
Shark	69.8 ± 0.11	25.3 ± 0.33	2.0 ± 0.11	5.1 ± 0.15
Tilapia	70.9 ± 0.08	21.7 ± 0.05	4.8 ± 0.38	33.5 ± 0.70
Sardine	67.4 ± 0.28	22.5 ± 0.24	6.2 ± 0.30	51.4 ± 2.35
Anchovy	74.8 ± 0.11	19.8 ± 0.18	3.3 ± 0.08	66.3 ± 2.56
Silverbelly	74.0 ± 0.11	20.5 ± 0.13	1.9 ± 0.06	29.0 ± 2.0
Shrimp	75.0 ± 0.15	19.9 ± 0.30	3.3 ± 0.06	51.2 ± 2.3

Table 6. Protein, fat and calcium content of cooked fish flesh and protein content of cooking water.

Variety	Protein (g/kg whole fish)	Protein of cooking water (g/kg whole fish)	Fat (g/kg whole fish)	Calcium (mg/kg whole fish)
Skipjack tuna	121.1	10.0	11.4	80
Yellowfin tuna	138.9	10.8	18.4	–
Snapper	98.2	11.2	18.5	138
Shark	114.7	14.3	8.9	23
Tilapia	88.0	9.5	19.5	136
Sardine	129.8	10.4	35.7	296
Anchovy	106.5	10.8	17.8	357
Silverbelly	75.7	7.4	7.2	107
Shrimp	69.5	16.3	11.5	178

Table 7. Average daily per capita consumption of protein, fat and calcium through fish, 1983.

Group	Total fish landed (t)	% landed	Protein (t)	Fat (t)	Calcium (g)
A 1 Skipjack tuna	14,195	6.5	1,861	161.8	1,136
A 2 Tuna and others	20,210	9.2	3,025	371.9	1,617
B 1 Trevally	8,583	3.9	939	158.8	1,184
B 2 Other demersals	23,566	10.7	2,578	476.0	3,252
C 1 Silverbelly	27,530	12.5	2,288	198.2	3,800
C 2 Other small pelagics	59,025	26.8	7,588	1,573.0	19,240
D 1 Shark	8,861	4.0	914	78.7	204
D 2 Skates and rays	10,306	4.7	761	91.7	237
E 1 Tilapia and other freshwater species	36,063	16.4	3,173	703.0	4,904
F 1 Shellfish species	5,298	2.4	465	60.0	331
G 1 Others	6,450	2.9	748	110.8	1,006
Total	220,087	100.0	24,320	3,984.0	36,911
Ave. intake/day/person at a mid-year population of 15 million	40.2 g		4.44 g	0.73 g	6.7 mg

Postharvest Histamine Formation in Dolphinfish in Southern Taiwan

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Abstract

Postharvest histamine content and microbial count of dolphinfish *(Coryphaena hippurus)* stored at various temperatures and periods were evaluated. Results show that the histamine content (HC) of a live and a dead dolphinfish caught from the sea of southern Taiwan and two samples iced commercially for 20 hours were 16, 20 and 35 mg%, respectively. The HC of the fishes chilled at 5 and 10°C for six days and the fishes frozen at -20°C for 14 days did not increase rapidly in the initial several hours but increased to a level of 100 mg% after 20 hours. Although the microbial counts of the fishes stored at 30°C for 12-16 hours increased to 10^6 CFU/gm, the HC did not increase and remained the same as the original level of the iced samples. On the other hand, the histamine formation in fishes treated with antibiotics, citric acid and acetic acid (pH 5) solution before storage at 15-20°C for 20 hours was inhibited significantly. The histamine formation rate is suggested as the function of free histidine content, pH of flesh, storage temperature, microbial count and specific surface area exposed to the atmosphere.

Introduction

The growth of histamine-forming bacteria (HFBs) on tuna, mackerel, dolphinfish *(Coryphaena hippurus)* and other fishes can lead to generation of histamine which can cause food allergy and idiosyncrasy when the histamine level increases to 100 mg% (Federal Register 4 September 1982). Freshly caught dolphinfish contains little histamine but possesses large amounts of free histidine. When the dolphinfish are spoiled by the growth of bacteria, such as *Proteus morganii, Enterobacter aerogenes, Klebsiella pneumoniae* and *Edwardsiella* sp. (Arnold et al. 1980; Niven et al. 1981; Okuzumi and Awano 1983; Yamanaka et al. 1984; Okuzumi et al. 1984a and 1984b; Frank 1985) the histamine would be decomposed by the decarboxylase. Recently, the FDA established a histamine level in tuna at 50 mg% as the health hazard level. The frozen dolphinfish fillet is an important export to USA from Taiwan. To understand the optimal postharvest treatment of dolphinfish, this experiment studied: (1) the histamine formation rate in the dolphinfish under different temperatures, and (2) the development of appropriate technology for fishing boats in developing countries and areas with poor chilling equipment by spraying with, or immersion into, acidic buffer solutions as antimicrobial agents to slow down bacterial growth and decrease histamine levels.

Materials and Methods

Dolphinfish, 2-3 kg each, caught live from the southern sea of Taiwan near the ports of Kaohsiung and Cheung-Kung were iced immediately on board the fishing boat with crushed ice. Fish were chilled to a low of 5°C within 1 hour and were carried to the laboratory within 6-20 hours. Individual fish were placed in separate polyethylene bags and incubated in temperature-controlled incubators for the desired period at 5, 10, 15, 20 or 30°C. Three fishes were treated for each temperature-time combination. About 2 x 2 x 2 cm flesh cubes with skin were sampled with sterilized knives from dorsal, ventral and tail tissue of each treated fish for measurement of histamine content and microbial counts.

For microbial counts, sampled flesh cubes, about 5 g, were homogenized with 1 part sterile 0.8% NaCl solution; aliquots of 0.1 ml were inoculated on triplicate plates of nutrient agar medium and kept at 35°C for 48 hours in an incubator before colonies were counted.

To measure histamine content, sampled flesh cubes, 5 g, were homogenized with 40-ml de-ionized water and 5 ml 1% trichloroacetic acid (TCA) and filtered. An aliquot of 1 ml of filterate was neutralized to pH 4.5-4.7 with 1% NaOH solution, to which was mixed 1 ml of 0.4 N acetate buffer (pH 4.6). One gram of amberlite CG-50 (100-200 mesh) ion-exchange resin suspended in 1 ml 0.2 N acetate buffer (4.6) was slurried into a 1.2 cm (internal diameter) chromatography column. The column was washed with 80 ml of acetate buffer to remove interfering substances and the histamine was eluted with exactly 8 ml 0.2 N HCl solution. A similar volume of 1.0% TCA was treated in the same manner to act as a blank. The eluate was mixed with 1.5 N $NaHCO_3$ to neutralize the pH, added with de-ionized water to a total of 10 ml, 2 ml of which was added with 2 ml of chilled diazo reagent, mixed and allowed to stand for 10 min. prior to absorption measurement at 510 nm using distilled water as the reference. Histamine content was calculated from the standard curve and dilution coefficient in mg% (Association of Official Analytical Chemists 1980).

To measure free amino acids, dorsal flesh 10 g was homogenized with 80 ml de-ionized water and 5 ml 20% TCA solution, filtered and measured with amino acid analyzer.

Results and Discussion

Fresh dolphinfish taken to the laboratory contained 8-25 mg% of histamine. Live and dead dolphinfish from the sea contained 16 mg% and 23 mg%, but when iced commercially for 20 hours levels increased to 20 mg% and 35 mg%, respectively. Preliminary experiments revealed that the dolphinfish treated with 250 mg% of ampicillin and chlortetracycline solution on the surface of fish and frozen or stored at 5, 20 and 35oC for 4-14 days did not increase in histamine content (Fig. 1). Thus, it can be proved that microorganisms play important roles in histamine formation from histidine.

Fig. 2 shows the rate of bacterial growth and histamine formation of dolphinfish at temperatures of 5, 10, 20 and 30oC at 0-140 hours. Although the microbial counts of the samples stored at 5 and 10oC for 120 hours increased to 10^6-10^7 CFU/g, the histmine content did not increase. In samples stored at 15oC for 70 hours, the bacterial count increased to 5 x 10^5 CFU/g and histamine began to increase to maximum (150 mg%) at the 88th hour. In samples stored at 20oC, the histamine content increased rapidly in the initial hours to a level of 100 mg% after 20 hours. Microbial counts of fishes stored at 30oC for 12-16 hours increased to 10^6 CFU/g and the fish were spoiled, but histamine content increased more slowly and remained the same as the original level of the iced samples.

The results reveal that the main decarboxylase producing bacteria belong to the psychrophilic type. The effect of spoilage of mesophilic bacteria on the muscle is more important than the postharvest histamine formation of psychrophilic bacteria in dolphinfish. Arnold and Brown (1978) classified the histamine-producing bacteria into two types. Type I bacteria produced histamine very fast at 20-25oC when pH is 5 but slower at pH 7. Type II bacteria produced histamine rapidly at pH 6-7 at 20-25oC. The main HFB at 20oC is *Proteus morganii* which does not produce histamine at temperatures higher than 35oC. Arnold et al. (1980) reported that *P. morganii, P. vulgaris* and *Hafnia alvei* produced more histamine in anaerobic than in aerobic conditions. The histamine-forming bacteria from *Scomber japonicus* produced histamine at 5-20oC (Okuzumi et al. 1981). However, Yoshinaga and Frank (1982) reported that the HFBs are *Clostridium perfringens, Enterobacter aerogenes, Klebsiella pneumoniae, Proteus mirabilis* and *Vibrio alginolyticus* and the optimal temperature for histamine formation is 38oC. The HFBs in fresh fish at 20oC are *P. morganii, P. vulgaris, Hafnia alvei, Citrobacter* spp., *Vibrio* spp. and *Aeromonas* spp.; *P. morganii* are found most frequently during the whole year and can grow in temperatures of 5-30oC (Okuzumi et al. 1984a, 1984b).

Yamanaka et al. (1984) revealed that the higher the temperature the more histamine is produced by HFBs. The main HFBs in the early spoilage stage are identified as psychrophilic and halophilic, but the mesophilic bacteria dominate in the later stage. The decrease of the histamine content of dolphinfish stored at 15oC for 90 hours from its maximum concentration may be due to the decomposition of diaminoxidase (histaminase) (Douglas 1975). Arnold et al. (1980) found the same phenomenon in histamine of skipjack tuna and reported that *P. morganii* lost its histamine-forming ability on the third day in contrast with *P. vulgaris* and *Hafnia alvei* because the histaminase could be formed from *P. morganii.*

The growth of microorganisms was reported to be inhibited by acids (Blocher and Busta 1983). To ascertain this, fresh fish in this experiment were treated by spraying or immersing them in citric or acetic acid. Microbial growth was inhibited and the histamine formation rate decreased to one-fifth of the control at the same storage temperature of 20oC (Fig. 3), while the critical period for histamine at 100 mg% extended from 20 to 70-90 hours (Fig. 4). Results show that immersion is more effective than spraying, probably because of the contact of bacteria with the acid.

Most bacteria do not grow well in an acidic medium; the HFBs grow well in pH 5.5-6.5 (Arnold and Brown 1978).

Rosenthaler et al. (1965) reported that the optimal pH of decarboxylase from *Lactobacillus* 30a and *Micrococcus* spp. are 4.8 and 5.8, respectively. A similar report showed that N-group bacteria except *Pseudomonas, Vibrio* and *Moraxella* can tolerate the low pH of 4.5 and grow. It seems that the HFBs are not inhibited by the acidic solution but the decomposition of tissue would be slowed down due to low pH. The intolerant bacteria are inhibited, and less histamine diffuses out of the tissue as substrate for decarboxylation. If this is true, the rigidity of the fish flesh is the other factor that influences the histamine-formation rate. It can be concluded that acidification treatment is valuable for postharvest preservation on fishing boats with poor chilling equipment as is common in developing countries.

From the kinetic view of the decarboxylation of histamine, there are three main factors that can influence the equilibrium of reaction, i.e., the concentration of histidine, the substrate; the activity of decarboxylase, the catalyst; and the temperature for bacterial growth and enzymatic reaction (Fig. 5). The histidine content of fresh dolphinfish measured in this experiment was 611.1 mg%, higher than in other strong histamine producers such as 595 mg% of juvenile mackerel (Sakaguchi et al. 1984) but lower than 1,125 mg% of yellowtail. Ferencik (1970) concluded that the minimum histidine concentration required for decarboxylase activity appeared to be 100 to 200 mg%. This study found that the histamine content of the dead dolphinfish (30.9-55.4 mg%) from the sea is higher than that of the live one (18.0-20.7 mg%) when both are iced for 20 hours.

That the higher histidine diffusivity of autolyzed or microbially destructured tissue would enhance the histamine-formation rate is proved. The concentration of decarboxylase would increase the histamine-formation rate as the enzymatic source (HFBs) increased when the special bacteria species had propagated at their optimum pH (5.5-6.5) and optimum temperature (15-25°C) on an autolyzed flesh which was rich in free amino acids. The reason for this is that the optimal reactive temperature of decarboxylase *(from P. morganii)* is about 37°C (Eitenmiller et al. 1982). The higher temperature would enhance the histamine-formation rate in the later postharvest period. The storage temperature is thus a critical factor that influences the formation of histamine in dolphinfish. Rapid and uninterrupted refrigeration after catch is necessary for postharvest preservation of dolphinfish.

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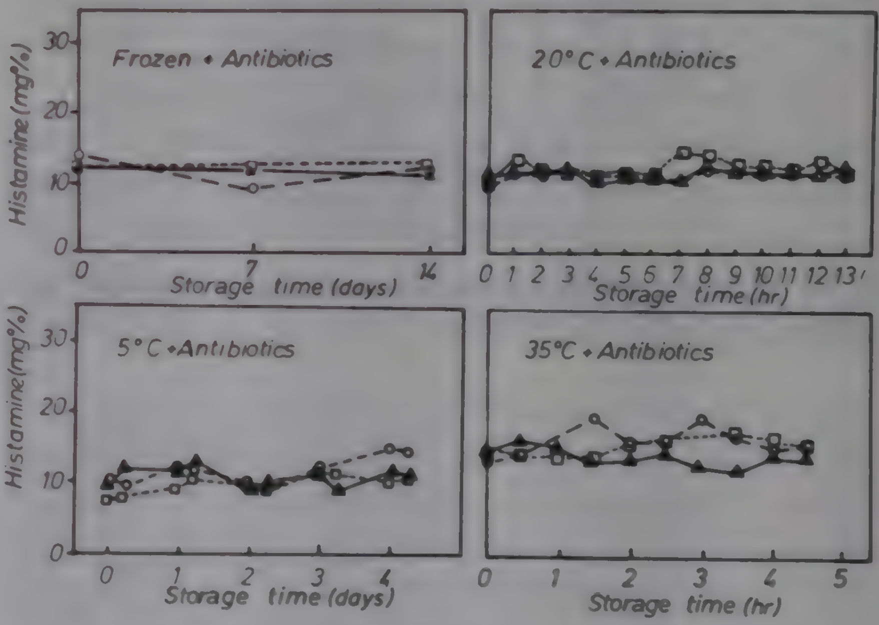

Fig. 1. Effects of antibiotics (ampicillin and chlortetracyclin) on the inhibition of histamine formation in dolphinfish. (□; dorsal, ▲; tail, ○; ventral).

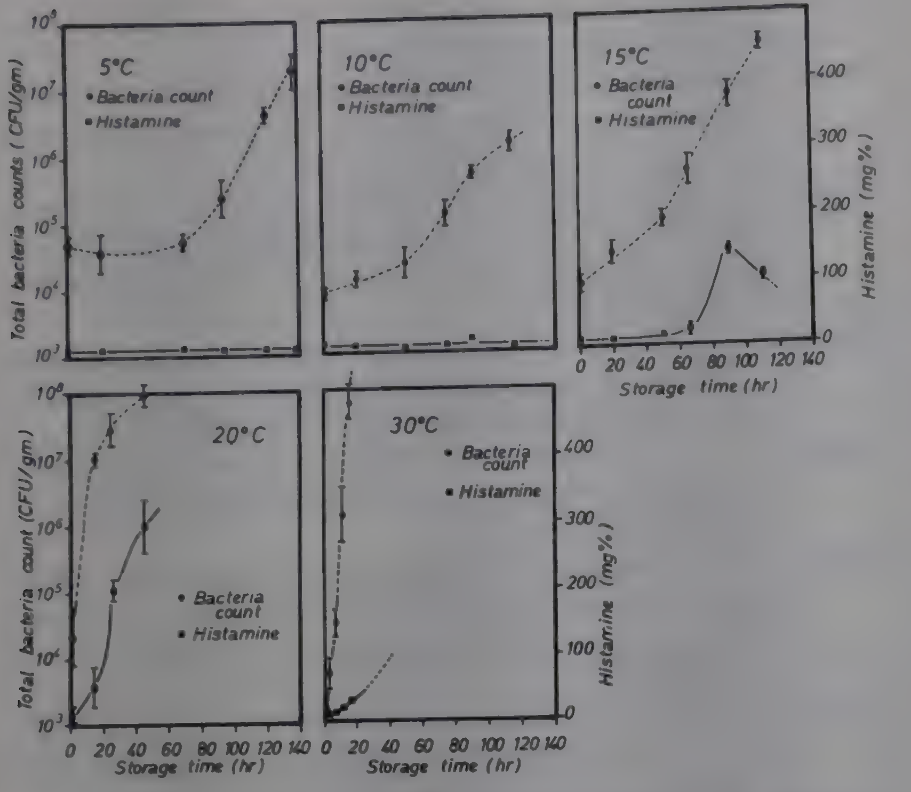

Fig. 2. Effects of temperature on bacterial growth and histamine formation in dolphinfish.

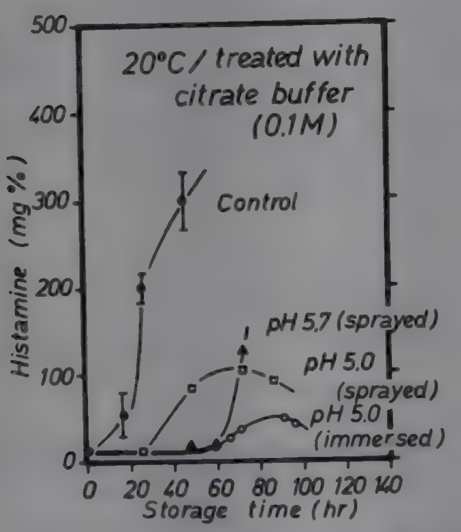

Fig. 3. Rate of histamine formation inhibited by treatment with citrate buffer solution at pH 5.0.

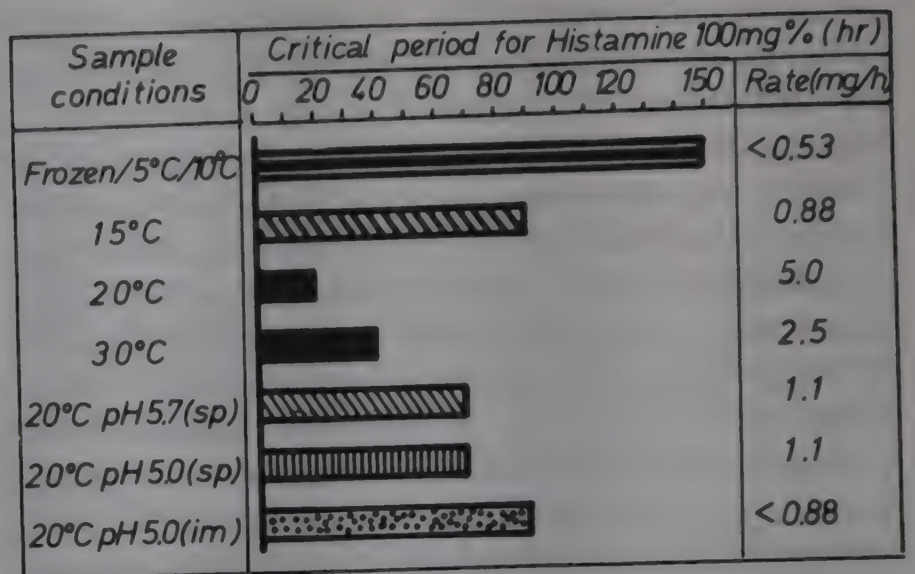

Fig. 4. Comparison of the histamine forming ability in dolphinfish under different conditions (sp = spray; im = immersion).

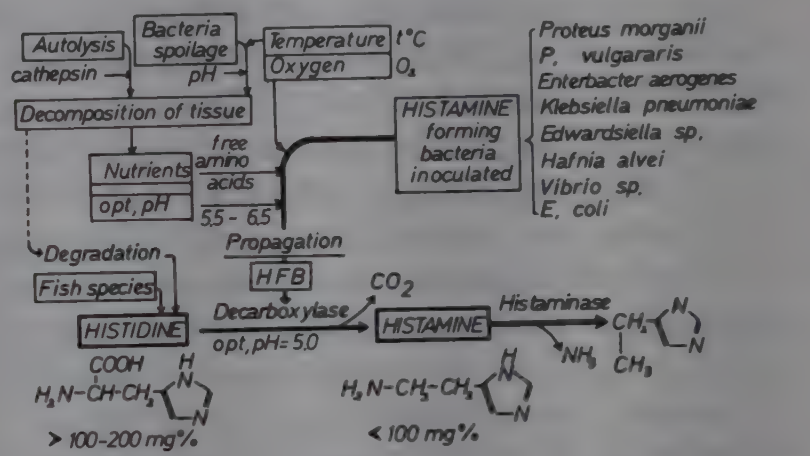
Autolysis
cathepsin
Bacteria spoilage
pH
Temperature t°C
Oxygen O₂
Decomposition of tissue
Nutrients
opt, pH
free amino acids
5.5 - 6.5
HISTAMINE forming bacteria inoculated
Proteus morganii
P. vulgararis
Enterbacter aerogenes
Klebsiella pneumoniae
Edwardsiella sp.
Hafnia alvei
Vibrio sp.
E. coli
Degradation
Propagation
HFB
Fish species
HISTIDINE
Decarboxylase
opt, pH = 5.0
CO₂
HISTAMINE
Histaminase
NH₃
> 100-200 mg%
< 100 mg%

Fig. 5. Overall view of histamine formation in fish muscle tissue.

Improved Bycatch Utilization in Thailand

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Abstract

Bycatch landing in Thailand has increased from 787,000 t in 1980 to 803,000 t in 1984 accounting for 40% of total marine production. Increased landings, in combination with the overexploitation of marine fisheries and declaration of the 200-mile exclusive economic zone of the neighboring countries have had a disastrous effect on domestic fish supply in Thailand. Bycatch and other underutilized species need to be better utilized for human consumption. Problems encountered in utilization of such species include poor quality of fish upon arrival at the landing places, small size and variation in shapes and species which have different flesh characteristics. Because of limitation in product storage life of bycatch handled onboard, sorting and icing should be confined to the last two weeks of the trip lasting 22 days. Studies on yield and storage life of minced sorted bycatch show that approximately 50% is fit for human consumption. The storage life of minced bycatch at 7°C is seven days; mince washed with diluted salt solution (0.2-0.3) has a 13-day storage life. A number of products from bycatch have been developed. Also, its potential for industrial processing is demonstrated.

Introduction

Total annual fish production of Thailand has fluctuated from 1.51 to 2.26 t x 10^6 over the past decade. The figure for 1983 of 2,225,433 t is only slightly above the 1977 figure of 2,189,907 t (see Table 1). Since the introduction of the otter board, trawler bycatch landing has increased to 803,000 t in 1983 comprising around 40% of the total marine fisheries production. Bycatch is mostly converted to fishmeal for animal feed. A great amount of pelagic fish, such as sardines and many other low-value fish species as well as the undersize fish such as small bonito, are also used for fishmeal production.

The production from the sea has reached the maximum sustainable yield and increase in bycatch landing has had a disastrous effect on domestic fish supply. The Government has realized the problem and has encouraged the expansion of aquaculture, as well as the utilization of fish bycatch and underutilized species.

Bycatch for Human Consumption

Fish bycatch is a mixture of small fish over 10-15 cm caught by otter board trawlers, pair trawlers and push nets. It mostly comprises juveniles of economic demersals and pelagics, as well as invertebrates. In the past decade, it made up 37-55% of the total marine production. Meemeskul (1985) reported in a survey by the government trawler, Pramong 2, in the Gulf of Thailand that 40% of the bycatch are juvenile commercial species. Bycatch obtained from commercial trawler fishing out of the Gulf of Thailand consists of 55% young economic species and 43% trash fish (Eiamsa-ard, unpublished data). The dominant species are threadfin bream *(Nemipterus* sp.*)*, goatfish *(Upeneus* sp.*)*, lizard fish *(Saurida* sp.*)*, trevally *(Caranx* sp.*)* and pony fish *(Leiognathus* sp.*)*.

Pelagic fish, in many cases, are sold as trash fish for fishmeal production. Their total production was 5,671 t in 1971 and 512,603 t in 1983. Pelagic fish resources in the Gulf of Thailand and the Andaman Sea are fully exploited.

The restrictions on number of trawlers and size of mesh to protect juvenile species are underenforced. Consequently, improvement will have to come in the form of better utilization of the catch, reduction of wastage and losses, greater efficiency and productivity on all levels of fisheries and development of new products of higher value.

Supply of fresh raw material is a problem if bycatch is to be used in commercial scale. Sorting, heading and gutting are labor intensive. Supichayangure (1985) demonstrated that improvement of handling bycatch onboard can be done by sorting during towing of each catch. Economic species are usually sorted onboard and kept for 15 days in ice. Up to 57% of the catch can be sorted out and sold at higher value. The price difference between sorted and mixed bycatch is not distinct. It is believed that the price will increase when its utilization is encouraged. Minced bycatch is 10 baht/kg (US$0.40); filleted fish cost 13-18 baht/kg. Minced yield is approximately 24% (Yamprayoon 1985).

Minced Fish from Bycatch

A study on bycatch use in the form of minced fish product was supported by the International Development Research Centre of Canada. Fishball, a popular gel product processed widely in Thailand, was chosen. The fundamental steps involved grinding the meat with sodium chloride to form a gel. Salt is used to increase ionic strength of the mince and dissolve actomyosin in fish muscle to form a sol. Heating of actomyosin sol until it reaches 90°C produces a network structure with an elastic texture. The fish must contain protein suitable for gel formation, kept at low temperature but not frozen and processed as soon as possible after landing.

A survey by SRG (1978) revealed at least 40 fishball factories in the Bangkok metropolitan area alone, most relying on traditional methods. At least 64 factories outside Bangkok utilize 3,466 t of raw material annually (Department of Fisheries 1986). Common species used are shark, barracuda, bigeye, eel, which are filleted. Since 1980, the manufacturers have been faced with the problem of price and supply of raw material.

Last-day bycatch produce was also considered. Species were sorted and kept in ice. Storage life in ice varied from species to species at 9-15 days. Gel strength of fishball produced from stored bycatch kept in ice for 10-12 days is fairly good (Suwansakornkul 1983). Ice retards degradation of salt-soluble protein responsible for gel formation.

Fig. 1 presents the method for fishball production. Fish must be kept cool at a temperature not exceeding 10°C. Grinding temperature should be controlled to prevent denaturation of protein. Suwanrangsi and Kiatkungwalkrai (1983) recommend a double step heating. When the gel passes the 60°C mark, part of it is destroyed due to alkaline protease which is active at 60-70°C (Suzuki 1981).

Flat fish, flathead, threadfin bream and goat fish sorted from ten species in the bycatch have the suitable flesh characteristics for raw material (Suwanrangsi and Kiatkungwalkrai 1983). The fishball produced was acceptable but did not compare with the commercial produce in texture and appearance.

Improvement of gel strength (elasticity) of products can be made by leaching or washing the mince with diluted salt solution. The method has shown improvement both in texture characteristics and color. Minced fish is washed in 0.2% and 0.3% at pH of 6.5-7 for 15 min. Soluble protein which interferes the gel formation is washed away as well as blood. Leaching causes the removal of fat from fatty mince up to 70% (Suwanrangsi 1985). The pilot-scale production showed that leaching does not have a distinct effect on improved gel texture. Leaching lessens the interference but does not affect the amount of gel protein.

Gel-forming ability of bycatch varied among species (Suwanrangsi and Kiatkungwalkrai 1983). Yamprayoon (1985) showed that the texture quality of fishball also varied according to species composition of bycatch.

Minced bycatch can be kept for seven days at 7°C. Storage life could be extended to 13 days if the mince is leached with salt solution. Fishballs produced from non-leached mince have poor quality on the first day; fishballs from leached mince have good and acceptable texture quality for up to nine days.

The price of the sorted bycatch should fall between the price of bycatch and economic species. At least 1 t/day of bycatch is needed by factories. Minced bycatch should be frozen for long-term use. The technology of Surimi production could then be applied to maintain the gel-forming ability of frozen minced fish. In the process, minced meat is washed at control pH at least three times. Sugar alcohol such as sorbitol and manitol and polyphosphate are added. Frozen minced fish block (Surimi) has a storage life of not less than one year without changes in texture quality.

Other Promising Products from Bycatch

Fish protein concentration (FPC type B) is a high protein product with the minimum content of 60% (Protein Advisory Group specifications). Yamprayoon and Kiatkungwalkrai (1983) have developed a processing technique to improve the sandy texture and water absorbency of the product. FPC produced from bycatch has met the standard requirement of the Protein Advisory Group. Yield of FPC is 4.55:1. The FPC is well accepted in the market.

Fish satay is another bycatch product using low-cost fish, mostly lizard fish, which has low gel-forming ability. It is a minced dried fish product containing 14.0% protein, 10% fat, 64% carbohydrate and 12% moisture. This type of product does not require elasticity. The processing involves deboning the fish, mixing with salt, sugar, flour and sesami seed, spreading into a round sheet, drying for 4-5 hours and then deep frying. The product can be kept for at least five months. Due to its high protein and calories, the Department of Fisheries encourages the use of this product to alleviate malnutrition especially in school children. Fish satay is produced also for export.

Bycatch can also be used in canned products because of the long storage life. Local recipes have been used to appeal to a wider circle of consumers.

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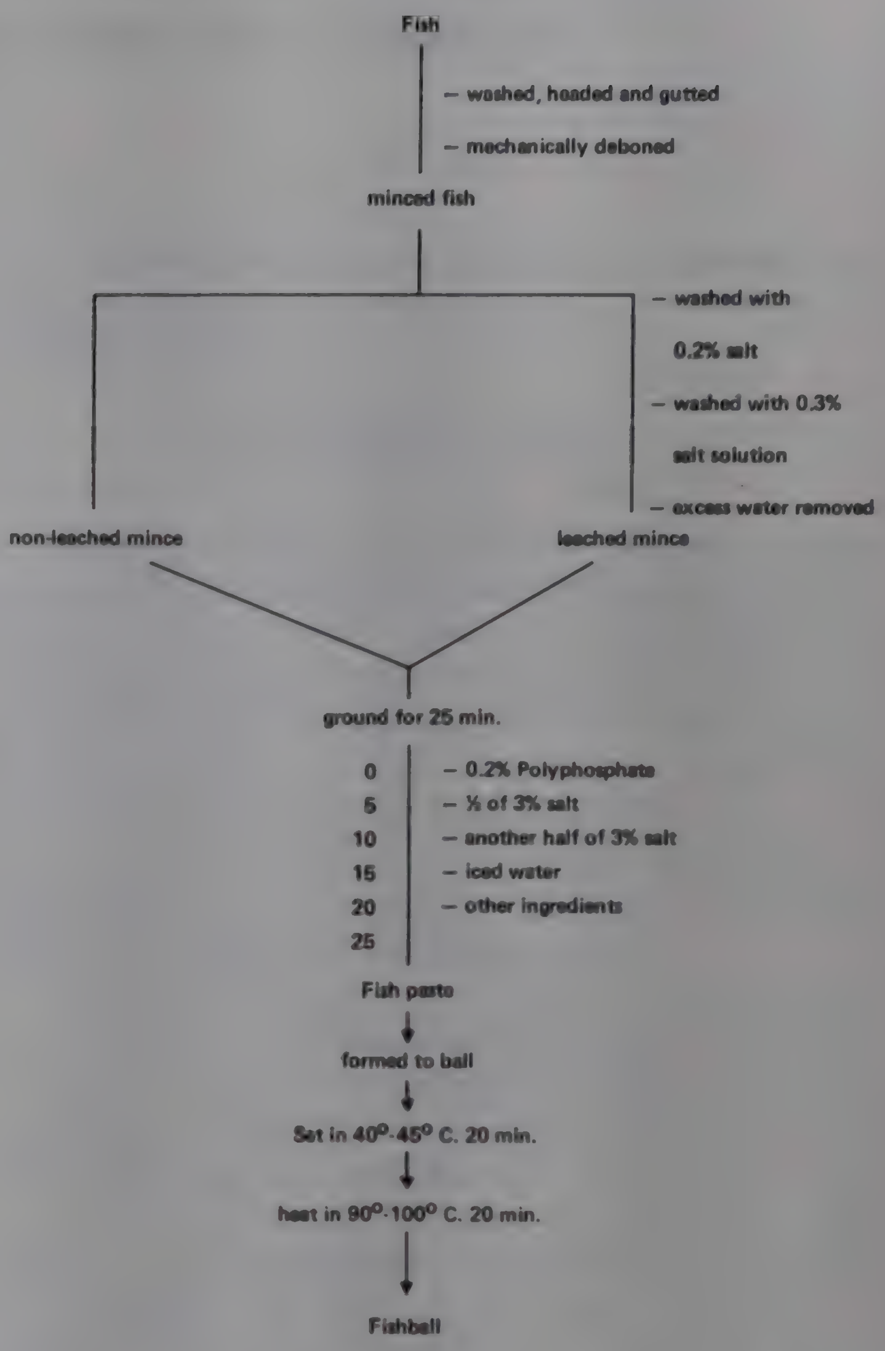
Fish
– washed, headed and gutted
– mechanically deboned
minced fish
– washed with 0.2% salt
– washed with 0.3% salt solution
– excess water removed
non-leached mince
leached mince
ground for 25 min.
0
5
10
15
20
25
– 0.2% Polyphosphate
– ½ of 3% salt
– another half of 3% salt
– iced water
– other ingredients
Fish paste
formed to ball
Set in 40°-45° C. 20 min.
heat in 90°-100° C. 20 min.
Fishball

Fig. 1. Fishball production.

Table 1. Production of Thai fisheries, 1971-1983 in tonnes. (Source: Fisheries Statistics of Thailand, 1971-1983).

		Freshwater fish		Marine fish		Bycatch	
Year	Total production	Quantity	% of Total catch	Quantity	% of Total catch	Quantity	% of Marine production
1971	1,587	117	7.36	1,470	82.64	655	44.57
1972	1,679	131	7.82	1,548	92.18	719	46.45
1973	1,678	140	8.39	1,538	91.61	804	52.31
1974	1,510	158	10.52	1,351	89.48	690	51.01
1975	1,555	160	10.33	1,394	89.64	634	45.53
1976	1,699	147	8.67	1,551	70.86	620	39.99
1977	2,189	122	5.59	2,067	94.41	836	40.46
1978	2,099	141	6.74	1,958	93.26	847	43.28
1979	1,946	133	6.84	1,813	83.16	784	43.25
1980	1,793	144	8.09	1,647	91.91	786	47.75
1981	1,989	164	8.27	1,824	91.73	796	43.67
1982	2,120	133	6.30	1,986	93.70	812	40.91
1983	2,225	155	6.89	2,099	93.11	803	38.25

Effect of Raw Material Freshness on the Quality of Smoked Tilapia *(Oreochromis niloticus)*

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Abstract

Brackishwater tilapia *(Oreochromis niloticus)* was prepared for smoking immediately after harvest (0 hour) and after holding for 3, 6, 9, 12 and 15 hours at ambient conditions (≈29°C; 71% RH). Quality assessment of the smoked products was done through sensory and physicochemical tests (determination of water activity, total volatile nitrogen, moisture and salt contents). Results showed that tilapia is considered acceptable for smoking even after 15 hours at ambient temperature. Delays in processing up to 9 hours gave good quality smoked products.

Introduction

Recent reports on the economics of tilapia culture stress demand-related problems including poor quality and perishability (Smith et al. 1983). Improvement of commercial handling methods is the most effective way to avoid wastage and to have products of better quality and nutritional value.

Studies by the Department of Fish Processing Technology at the UPV College of Fisheries on the spoilage patterns of this fish at ambient conditions (Estrada et al. 1984) and stored in ice (Abduhasan 1985) showed that tilapia is still acceptable even after 15-18 hours at ambient conditions. When stored in ice, it keeps for 26, 20 and 16 days if icing is delayed for 0, 4 and 8 hours, respectively. Fish dealers claim that after only a day's storage in ice, the color of tilapia fades, lowering its market value.

This preliminary investigation was designed to find out the maximum time that this species can be kept at ambient conditions and still be acceptable for smoking. Fish were delayed or held at prevailing conditions (≈29°C and 71% RH) for 0, 3, 6, 9, 12 and 15 hours before smoking. Quality was assessed by a taste panel and physicochemical tests.

Materials and Methods

Tilapia samples *(Oreochromis niloticus)* were obtained from a brackishwater pond in Bulacan during the month of May. The fish were iced immediately after harvest, packed in styrophore box and remained in this condition for approximately three hours until they reached the Department of Fish Processing Technology laboratories in Quezon City, Philippines. They were then removed from ice, washed with tap water and maintained moist while exposed to ambient conditions (≈29°C and 71% RH) throughout the sampling period.

Fifty to sixty pieces of fish, each 16-18 cm long and 200-250 g, were withdrawn every three hours, washed and prepared for smoking. Preparation involved gutting, eviscerating, washing, brining (20 min. in 20% salt solution) and dipping in boiling weak brine (5-10%) for 8-10 min. The precooked fish were kept at 10°C while waiting for the sampling period to be completed. Smoking of all samples was done at the same time inside a mechanized smokehouse (Afos Torry Kiln Model 20) at 40°C for 30 min. then at 83°C for a total of 2 hours.

Samples were presented raw and fried to a panel of 30 to 40 consumers for acceptability testing. Scorecards with a scale of 1 to 6 (very poor to excellent) and 2 as the rejection point were used in rating. Analysis of variance and Duncan's multiple range test were used to gauge significant difference among samples (Gatchalian 1981).

Moisture content and water activity (a_w) were measured by using OHAUS infrared moisture balance and Lufft's a_w meter, respectively.

Salt content was analyzed using a modified Volhard method and total volatile nitrogen was determined by the Conway microdiffusion technique (1969).

Results and Discussion

Fig. 1 illustrates the results of the preference test conducted. Tilapia held at ambient conditions for 0, 3 and 6 hours did not differ significantly in terms of acceptability. Fish held for 6 and 12 hours also did not show significant difference although those held for 9 hours were significantly different at the 5% level. The samples held for 15 hours were the least preferred in all the parameters tested; those held for 0, 3 and 6 hours obtained higher scores.

Although flavor was unacceptable after 15 hours, the raw odor, salt flavor and texture of the products were still acceptable to the taste panel. Tilapia held for 15 hours were rejected due to strong rancid, stale and slightly sulfidy taste attributed to lipid oxidation from the fat content of 9.1-10.1% (Watanabe et al. 1980). Smoke did not mask the rancidity of the fish. Furthermore, the process could have increased its susceptibility to oxidation.

Table 1 presents the results of other tests. The salt content increased as delays were extended, although it was only on the 15th hour when the change was significant This could be partly accounted for by the fact that the raw material was in the postrigor state at this point. During this period, the water binding capacity of fish muscle decreases and salt uptake is faster (Voskresensky 1965). This would also explain the lower salt content of the smoked product from the raw material processed immediately after receipt at the laboratory (0 hour).

The moisture content and a_W of the products changed from 69 to 67% and 0.94 to 0.93, respectively. The decrease in these components may be due to the extended delays which made water diffusion from the fish more rapid.

The total volatile nitrogen (TVN) increased exponentially with values of 4.2 to 11.3 mg % (Table 2). The TVN content upon rejection was much lower than the reported freshness limit for other fish which is 30 to 40 mg % (Connell 1980). Estrada et al. (1984) reported the same low value of TVN upon rejection of tilapia at ambient storage. TVN is always associated with odor and these preliminary results showed that even if the TVN content of the smoked products were low, the odor was sulfidy and slightly putrid upon rejection which is typical of spoiled fish.

Acknowledgements

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Table 1. Proximate composition of tilapia *(Oreochromis niloticus)*.

% Moisture	% Protein	% Lipid	% Ash
71.6	14.7	9.4	3.6
70.1	14.4	9.5	3.9
70.6	14.0	10.1	3.9
70.9	14.7	9.1	4.1

Source: Watanabe et al. 1980.

Table 2. Salt (NaC1), total volatile nitrogen (TVN), moisture content and water activity (a_W) of smoked tilapia held at ambient conditions before processing.

Holding time (hr)	NaC1 (%)	TVN (mg %)	Moisture (%)	a_W
0	1.5	4.2	69	0.94
3	2.12	4.48	69	0.94
6	2.20	4.96	69	0.93
9	2.35	5.32	67	0.93
12	2.50	6.55	67	0.93
15	4.32	11.03	67	0.93

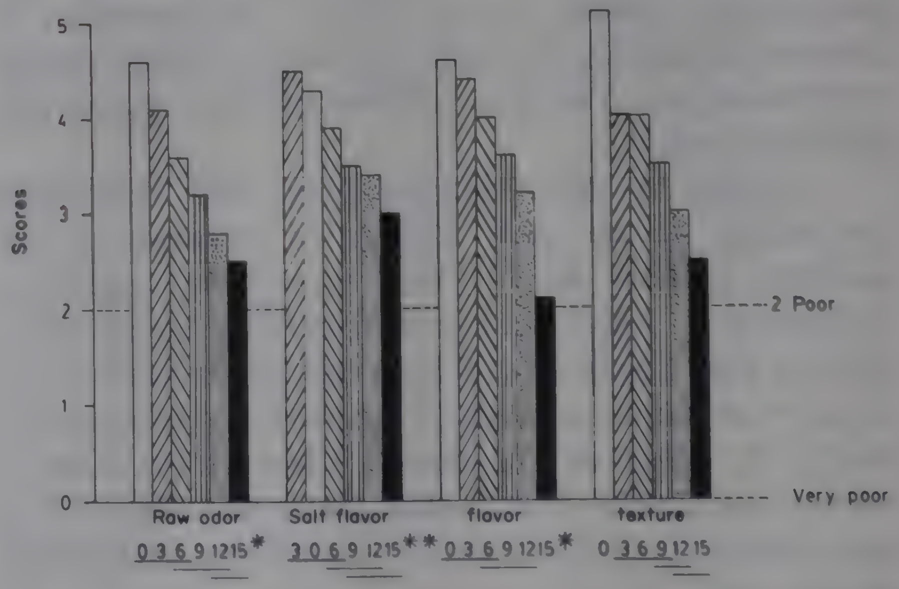

Fig. 1. Acceptability of smoked of tilapia with delays of 0-15 hours at ambient conditions before processing. Horizontal broken line shows the rejection level. Samples connected by a bar beneath the figure were not significantly different.

*Significant at 5% level.

**Significant at 1% level.

Production and Storage of Smoked Spanish Mackerel *(Scomberomorus commerson)*

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Abstract

A procedure for smoking Spanish mackerel *(Scomberomorus commerson)* of *tangigue*, using a mechanical smokehouse was developed. Changes in the physical, chemical and microbiological properties of the fish muscle during the smoking process were determined. Storage stability at refrigeration temperature (0-5°C) of vacuum and non-vacuum packed products was studied. There was no significant difference in the storage life between vacuum and non-vacuum packed smoked *tangigue*. Packaging this product hygienically in medium density polythene films is cheaper than vacuum-packaging. Smoked samples, both vacuum and non-vacuum packed, develop a more acceptable flavor after one week storage at 0.5°C. Storing smoked *tangigue* at refrigeration temperature packed in flexible plastic films, both under vacuum or not, results in products with no marked change in pH, moisture content and water activity after 30 days. Histamine increases in both packs although the concentrations after storage do not reach significant levels. There is no risk of growth of pathogenic microorganisms in smoked Spanish mackerel if one starts with very fresh fish and contamination during processing and handling is kept to a minimum. Storage temperature must also be near 0°C.

Introduction

Spanish mackerel, locally known as *tangigue*, is a species which is abundant in Philippine waters where the annual catch is approximately 20,000 t. The price of this fish ranges from US$1.50 to US$2.50/kg depending on the proximity to the source. This species is usally marketed fresh or iced and is used for sashimi and pickled recipes.

A smoked product with a high market value for both local and export markets was developed from different smoking procedures with varied brine concentrations, smoking time and temperature. From 100 kg of fish, 40-48 kg were obtained as fillets suitable for smoking, 35-40 kg for human consumption and the remaining 17-20 kg for animal feed.

This paper discusses the results of a study conducted on the smoked product stored at 0-5°C. A water vapor gas-impermeable film and a medium density polyethylene pouch were used for packaging. The objective was to market a product with a high consumer appeal and hygienic presentation.

Materials and Methods

Spanish mackerel caught off the Tayabas and Lamon Bays in Quezon Province, Philippines, were obtained through a fish dealer at the Farmer's Market in Cubao, Quezon City. The fish were bought whole (8 to 10 kg/piece), un-iced but chilled and transported to the Department of Fish Processing Technology laboratories.

The smoking procedure developed is as follows. Very fresh raw material is required, otherwise poor quality products are obtained. The fish were filleted, skinned and washed thoroughly. The time for brining varied according to the fillet size. The salt content of fillets measuring 25 x 9 x 4 cm and weighing 450 g saturated in brine for 30 min. was the most acceptable to the consumer taste panel. After brining, the fillets were washed in freshwater to remove surface salt and drained for one hour at 10 to 15°C to remove surface moisture and to equilibrate salt in the fish flesh. Fish were smoked for 8 hours at 45°C inside a mechanized kiln (Torry kiln model 20) after which they were cooled, packed and then stored at refrigeration temperature.

Fig. 1 outlines the storage experiments conducted on the smoked products. The samples were stored both as fillets and slices (around 3-mm thick) in a household refrigerator with temperature maintained at 0-5°C. One lot was heat sealed in polyethylene (0.003-mm thick) bag and another was vacuum-packed (using a MULTIVAC sealer), wrapped in flexible oxygen barrier film prepared from nylon/ionomer/PE copolymer layers with a WVTR of 0.3 g/100 in^2/24 hours and oxygen permeability of 0.37 cc/100 in^2/ATM/24 hours at 36°F. A polyfoam backing was provided in the pouches with sliced samples. Two or three packs of the samples measuring 14 x 18 cm and weighing approximately 100 g each were withdrawn every 3 to 4 days for analyses.

All samples were prepared in duplicate and tests done in two trials. At least two packs (chosen randomly) per lot were opened and composite sampling observed. For histamine determination, during the second and third trials care was taken to ensure that samples came from one fish and that the portion analyzed was uniform for all lots.

Chemical tests include: salt analysis by a modified Volhard Method; total protein determination by the Kjeldahl-Gunning method; and determination of histamine content through the fluorometric method of Taylor et al. (1978).

Moisture content was determined by direct reading using an infrared moisture balance (OHAUS) and water activity (a_W) was measured using Lufft's water activity meter.

Standard plate count was at 20°C. The number of organisms expressed as cfu (colony forming units) per gram of sample was determined following the spread plate technique on plate count agar (Oxoid).

Two media (Perfringens Agar and Cooked Meat medium, Oxoid) were used to check for *Clostridium botulinum* and *C. perfringens* spores.

A panel of 25 to 35 members assessed the acceptability of the samples. The choice of panelists was limited to possible consumers/buyers of the product. The samples were presented in a ready-to-eat manner, sliced thinly and chilled (approximately 20°C). Flavor (salt and smoke), texture and appearance, particularly color, were analyzed. Evaluation of stored samples was done by a regular panel of 5-8 members composed of teaching and research staff. They were asked to rate the products based on the same attributes as the consumer-type panel although during the first trial assessment was limited to appearance and odor. A scale of 1 to 6 (very poor to excellent) was used. Analysis of variance, t- and Tukey's tests were used to determine significant differences among samples (Larmond 1977; Gatchalian 1981).

Results and Discussion

The proximate composition of smoked Spanish mackerel is presented in Table 1. This species is classified as a low fatty fish. Thus problems of rancidity or fat oxidation during storage are minimal. Salt content was about 4%, the level most acceptable to the taste panelists; moisture content was around 66% and water activity (a_W), 0.94. Unless the products are stored at refrigeration temperature, bacteria will grow and spoil the product.

Table 2 shows how some parameters changed during the smoking process. Moisture content was reduced by brining and smoking. Salt had the effect of removing water from the fish flesh and fish were characteristically dried during smoking. There was little change in pH and histamine levels. Spoiled scombroid fish generally contain about 10-50 mg histamine per 100 g flesh, and can have as much as 1,000 mg per 100 g. The US FDA established a level of 20 mg per 100 g in tuna as representing health hazard (Wood and Bostock 1984). Fish implicated in outbreaks of food poisoning contain elevated histamine levels usually in excess of 100 mg per 100 g (mg %) of fish (Wood and Bostock 1984). Values presented from the smoked product were below these significant levels. The number of microorganisms were reduced by about 95%. Overall, no significant quality deterioration took place during the smoking process.

There was no difference in spoilage patterns between sliced and filleted samples, both vacuum and non-vacuum packed, although the former obtained slightly higher scores than the latter. Fig. 2 shows changes in the mean taste panel scores for the sliced smaples. In terms of appearance, odor and flavor, the vacuum-packed samples obtained higher scores. An interesting observation was that after almost a week of storage the flavor of the samples improved, hence the increase in the mean scores. The smoke and salt flavor imparted during the process must have fully diffused and equilibrated through the flesh. After 30 days, a decline in the scores was observed and samples were rejected based on flavor with the mean score of the vacuum-packed samples still slightly above the acceptable limit. The appearance of the samples was still acceptable despite rejection of taste. The golden brown color on the surface and creamy white inside remained the same. Odor was slightly sour to acidic and flavor less smokey, stale and slightly itchy. The texture of the vacuum-packed samples was softer, soggy and wet; those non-vacuum packed were more fibrous and slices tended to break easily. The resiliency or elasticity of the flesh was lost during storage. Statistically, the keeping quality of the two products packaged differently did not vary significantly. Both had a shelflife of 30 days at 0-5°C.

Table 4 shows the histamine accumulation in the smoked samples during the storage period. Histamine concentration increased in both packages, although the values did not reach toxic levels. Increase in histamine content may be related to the increase in the aerobic counts during storage. Vacuum-packed samples had lower microbial counts at the middle stage of storage, but in the end, the difference between the two was negligible. *Cl. botulinum* and *Cl. sporogenes* were not detected in the packs.

Other physical parameters like pH, moisture content and a_W of the samples did not change significantly during storage. This shows that the packaging materials used in the study acted as physical barrier for the evaporation of water from the packs. The constant pH could mean that the microorganisms in the packs were not acid-producers. If they were, the storage conditions inhibited their activity. The buffering capacity of the fish flesh might have also controlled the pH.

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Table 1. Proximate composition of smoked Spanish mackerel.

% Fat	1.37
% Protein	24.96
% NaC1	3.96
% Ash	2.31
% Moisture	65.50

Table 2. Changes in fish muscle during processing at 45°C.

Processing stages	Moisture (%)	pH	Histamine (mg %)	SPC (log cfu/g)
Raw material	75.0	5.37	0.54	4.94
Brined	69.5	5.27	0.57	3.76
Smoked	65.5	5.25	0.60	3.51

Table 3. Changes in histamine content (mg %) of sliced samples during storage at 0-5°C.

Storage day	Vacuum packed	Non-vacuum packed
0	1.120	1.120
14	2.185	2.980
30	3.187	4.200

Table 4. Changes in aerobic plate count (log cfu/g) of sliced samples during storage at 0-5°C.

Storage day	Vacuum packed	Non-vacuum packed
0	4.91	4.91
14	5.96	6.28
30	7.60	7.75

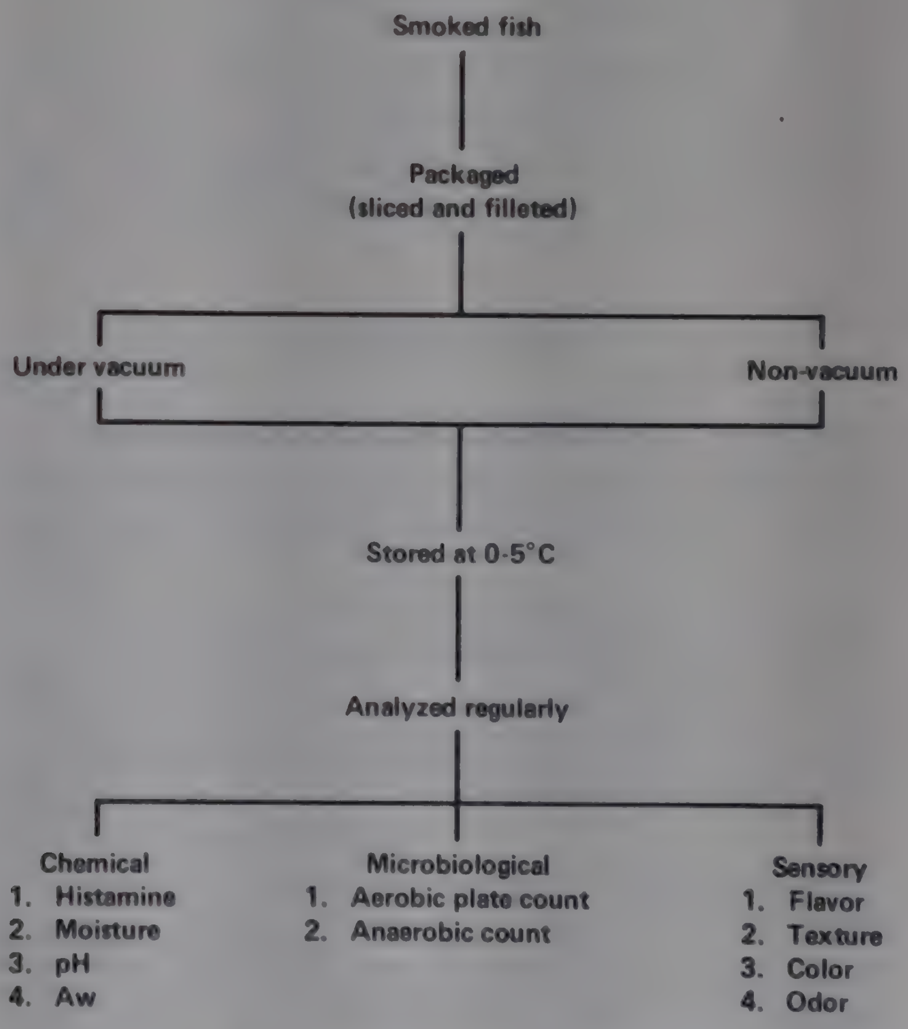
Smoked fish
Packaged
(sliced and filleted)
Under vacuum
Non-vacuum
Stored at 0-5°C
Analyzed regularly
Chemical
1. Histamine
2. Moisture
3. pH
4. Aw
Microbiological
1. Aerobic plate count
2. Anaerobic count
Sensory
1. Flavor
2. Texture
3. Color
4. Odor

Fig. 1. Schematic diagram of storage experiments conducted on smoked Spanish mackerel.

An Assessment of the Exploitation of Coral Reef Fishery Resources in Papua New Guinea

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Abstract

Past utilization of Papua New Guinea's coral reef resources has been low in comparison with other countries which possess similar resources. This does not appear to be due to resource limitation but to social and economic factors. Although the government has actively encouraged increased exploitation of the coral reef resources, finfish harvests have only increased slowly to an estimated 8,344 t/year of which 7,235 t is subsistence catch. The only industrial fishery in the coralline areas is a supportive live-bait fishery for a pole-and-line tuna fishery which has developed since 1970. This fishery specifically targets previously unexploited stocks of pelagic anchovies and sprats and caught on average 1,223 t/year during the first 11 years of operations. Approximately 420 t/year of benthic invertebrates such as pearl shells, spiny lobster and sea cucumber, are harvested each year for export. Annual harvests of pearl shells have declined markedly from a mean of 748 t/year during the 1950s to 333 t during the present decade. The reasons for this and other trends associated with the exploitation of organisms from coralline areas are discussed.

Introduction

The exploitation of coral reef organisms in Papua New Guinea (PNG) can be broadly classified into three categories: first, subsistence fishing for food that has occurred since pre-history (White and O'Connell 1979); second, commercial artisanal fishing for a cash return (Wright et al. 1983; Frielink 1983; Wright and Richards 1985; Lock 1986a 1986b, 1986c, 1986d); and third, a supportive live-bait fishery for pole-and-line tuna fishing (Dalzell and Wankowski 1980; Dalzell 1984). This paper reviews the present fishing practices in PNG's coral reef areas, estimates the size of their current yield and discusses the levels of exploitation of the reef resources with respect to social and economic factors.

The Coralline Areas of PNG

PNG consists of the eastern half of mainland New Guinea and the island groups that lie to the east and north in the Bismarck, Solomon and Coral Seas. Most accounts of PNG's coral reefs are unsatisfactory and in some cases erroneous. Whitehouse (1973) stated that the northeastern coast of PNG was devoid of active reefs. However, Kojis et al. (in press) found large areas of fringing reef along the northeastern coast of mainland PNG of a diversity and continuity comparable to the Australian Great Barrier Reef.

Frielink (1983) estimated approximate areas of coral reef to a depth of 30 m around the PNG coast from hydrographic charts. The estimates are given in Table 1 with estimates of shelf area calculated by Munro (1976). The greatest concentration of coral reefs is in the Milne Bay Province which has 1,287,000 ha or about 30% of the total. The Gulf and Western Provinces are virtually devoid of coral reef and are combined as a single area whilst the Torres Strait region of Western Province is listed separately in Table 1. Areas of high freshwater influx in the Gulf and Western Provinces, and the mouths of the Sepik and Markham Rivers on the north coast, are similarly devoid of reef-building corals.

Artisanal Fisheries

The subsistence catch in the coralline areas of PNG was estimated to be 7,235 t/year, based on population densities and consumption rates of fish given by Frielink (1983).

The volume of fish landings that pass through outlets other than Government Fishing Purchasing Centers (GFPCs) is unknown. The total annual catches of fish landed at the nine GFPCs within PNGs coralline areas are summarized in Table 2. Over this period, the total landed catch has increased by 63%.

Only two artisanal commercial reef fisheries in PNG have been studied in any detail. Wright and Richards (1985) investigated a lightly exploited reef fishery in the Tigak Islands of northern PNG, whilst Lock (1986a, 1986b, 1986c, 1986d) studied the intensively exploited reef associated fishery in proximity to the capital city Port Moresby. In both instances, fish are caught principally by gill netting and hand lining with spearing and trolling contributing lesser amounts to the catch. The composition of the catch of both fisheries is given in Table 3. Mullets (Mugilidae) made the largest contribution to the Tigak Islands fishery of which 19.1% was *Valamulgil seheli*. By contrast, Lethrinidae or emperors dominated the Port Moresby catch and accounted for about 29% of the total.

The catch rates for different artisanal reef fisheries in PNG are presented in Table 4. Wright and Richards (1985) estimated that the total yield of the Tigak Islands 20,765 ha of coral reef and associated environments was 4.2 kg/ha/year of which 0.63 kg was caught for subsistence purposes. Lock (1986c) calculated that yields for the various fishing grounds in the Port Moresby fishery varied between 3.5 and 83.0 kg/ha/year with a mean of 50 kg/ha/year. Lock ascribed these differences to variation in fishing effort and estimated that the maximum sustainable yield (MSY) for 11,600 ha of reef which made up the Port Moresby fishery was in the region of 70-75 kg/ha/year.

The Live-Bait Fishery

The domestic pole-and-line tuna fishery and supportive live-bait fishery in PNG commenced in earnest during 1971. The total catch of bait-fish from 1971 to 1981 was 13,451 t with a mean annual catch of 1,222.8 t. Fishing operations were suspended in 1981 and did not recommence until August 1984. Although bait catches were made at 11 sites in PNG's coralline regions, over 60% of the total catch came from two locations, the Ysabel Passage and Cape Lambert (Dalzell 1984).

Details of the species composition of the Ysabel Passage and Cape Lambert baitfish catches are given by Dalzell and Wankowski (1980) and Dalzell (1984). The catches of baitfish at the Ysabel Passage were dominated by two anchovies, *Stolephorus heterolobus* and *S. devisi*, and a sprat, *Spratelloides gracilis*. At Cape Lambert *Sp. gracilis* was virtually absent from the catch and the dominant species were *S. heterolobus*, *S. devisi* and the oceanic anchovy, *S. buccaneeri*, which was subdominant.

Surplus yield models (Schaeffer 1954; Fox 1970) could not be fitted to total catch and effort data from either the Cape Lambert or Ysabel Passage bait fisheries (Dalzell and Wankowski 1980; Dalzell 1984). Dalzell (1983), however, showed that data on combined catches of *S. heterolobus* and *S. devisi* could be fitted by a simple Schaeffer curve. The annual MSY for both species was estimated to be 0.61 t/ha of bait ground or 204 and 208 t at the Ysabel Passage and Cape Lambert, respectively.

Other Fisheries

Between 1950 and 1984, a total of 17,004 t of pearl shell was exported from PNG with a mean annual shell harvest of 485.8 t. A summary of pearl shell type and quality exported in five year groupings is given in Table 5. About 84% of the total shell harvest was trochus *(Trochus niloticus)*, with green snail *(Turbo marmoratus)* and black lipped pearl shell *(Pinctada margaritifera)* making up the remaining 12 and 4%, respectively.

Virtually all pearl shell production in PNG comes from the coralline areas but little is known about regional production or of the effects of sustained harvesting on the various mollusc populations. Glucksman and Lindholm (1982) suggested that overfishing of trochus and green snail has occurred in the past in certain areas such as the south coast of Manus. These authors based their conclusions on anecdotal information on yield and the small size of trochus and green snail shells from this area compared with shells from unexploited areas.

A total of 137 t dry weight of sea cucumber was exported from PNG between 1960-1984, with a mean annual production of 5.5 t. The figures for total production in five-year groupings are given in Table 5. Dry weight can be converted to approximate wet weight by multiplying by a factor of ten (Anon. 1979).

Ito (1983) and Ito and Selemet (1984) gave the only details of regional production of sea cucumber in PNG. Between 1982 and 1984, all sea cucumber production from the North Solomons Province came from three oceanic atolls, the Nuguria, Mortlock and Cartaret groups, and consisted solely of the teat fish *Microthele nobilis* (Ito, pers. comm.). There was a marked decline in production from the three atolls from 14.1 t in 1982 to 1.2 t in 1984. As 63% of the total harvest came from the Cartaret Islands, Ito and Selemet (1984) suggested that decline in production from this location may have been due to overfishing. The reduction in harvest from the Cartaret Islands was also accompanied by reduction of mean size.

The major crustacean fishery in PNG's coralline areas is the ornate rock lobster, *Panulirus ornatus*, at the extremes of the Gulf of Papua. At Daru and the northern Torres Straits in the west and Yule Island in the east, artisanal diver fisheries produced 386.6 t of lobster tails between 1973 and 1984 (Anon. 1985). *P. ornatus* makes an extensive spawning migration during August and September from the Torres Strait reefs, across the Gulf of Papua to the Yule Island reefs (Moore and MacFarlane 1984). Since 1973, this migration has given rise to a trawl fishery which by 1984 had caught 902.1 t of lobster tails (Anon. 1985).

Other edible crustaceans in PNG's coralline areas are rock lobsters such as *Panulirus homarus* and *P. versicolor*, mantis shrimps (Stomatopoda), reef crab *(Carpilius* *maculatus)* and hermit crabs *(Coenobita* sp.*)*. Wright et al. (1983) estimated that about 8.0 t of rock lobsters were harvested annually from the Tigak Islands reefs or a yield of 0.39 kg/ha/year.

Discussion

The current fish and invertebrate harvests associated with coral reefs in PNG are low compared with coral reef fisheries elsewhere. The total accountable catch of all organisms during 1984 from PNG's coralline areas, including the empirical subsistence estimate, was 8,344 t, or a yield from 4 x 10^6 ha of reef of 2.3 kg/ha/year. As some catches are not accounted for, such as market and store sales, this figure is an underestimate.

Munro (1978) estimated the yield of neritic pelagic and demersal fish and crustaceans from coral reefs and coralline shelf within the 200-m isobath in the Carribean to be 44 kg/ha/year. Wass (1982) determined that fishing in American Samoa occurred almost exclusively in depths less than 8 m, the majority taking place on the reef flat where the annual yield of all marine animals was estimated to average 266 kg/ha/year. The finfish component averaged 160 kg/ha/year with a range of 69-355 kg/ha/year for different areas fished by the villages in the study site. Alcala (1981) and Alcala and Luchavez (1981) reported yields of 80-237 kg/ha/year for reefs extending to depths of 60 m surrounding Apo and Sumilon Islands in the Philippines.

Wright and Richards (1985) suggested that the low yield of finfish of 4.17 kg/ha/year from the Tigak Islands was likely to be indicative of most reef fisheries in PNG. The high yield of 50 kg/ha/year observed by Lock (1986c) for the Port Moresby reef fishery is a combination of the high population densities of people there which represent a large concentrated market for fresh fish and that the villagers involved in fishing are full-time fishermen. These villagers are residents of a low, agriculturally poor island, and have few alternative means of acquiring cash or food.

Most coastal villages in PNG are not fishing villages and fishing is only one activity in village life (Tiller 1984; Wright and Kurtama, in press). Time is also devoted to the raising of food and cash crops such as cocoa and copra. When the buying price of copra is high, this can act as a disincentive to catch fish for a cash return (Anon. 1985). Further, the ready availability of cheap canned fish and meats reduces the need to go fishing, even for subsistence purposes (Tiller 1984).

The same factors are likely to apply to pearl shell and sea cucumber harvests, although these products command much higher prices than finfish. Further, the marketing infrastructure for such products has contracted since the 1950s; the number of traders willing to visit outlying productive areas has dropped because of high overheads (Glucksman and Lindholm 1982). Shell collection and distribution is now centralized in urban areas and production has declined.

Bait fishing during 1984 and 1985 in PNG waters was much reduced due to the smaller revitalized pole-and-line fleet. The mean number of vessels operating between 1971 and 1981 was about 45/year compared with nine fishing boats operating in 1985. As long as pole-and-line tuna fishing persists in PNG waters, baitfish will continue to be caught in the coralline areas of the country. However, the large-scale reduction of the fleet size between 1981 and 1984 is indicative of the effects of economic constraints on this industry discussed by Doulman and Wright (1983).

It appears from the foregoing that most of PNG's coral reef associated fish and invertebrates are only lightly exploited. Yields are effort limited rather than resource limited. The same is probably true of PNG's other coastal fisheries, particularly where artisanal fishing methods are used.

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Table 1. Estimated reef (Frielink 1983) and shelf (Munro 1976) areas of PNG Provinces.

Province/Area		Shelf area to a depth of 200 m (x 10^3 ha)	Reef area to a depth of 30 m (x 10^3 ha)
Torres Strait		3,378	1,042
Western/Gulf	(A, B)	4,578	–
Central	(C)	582	187
Milne Bay	(D)	4,115	1,287
Oro	(E)	1,652	517
Morobe	(F)	246	77
Madang	(G)	89	29
East Sepik	(H)	63	21
West Sepik	(I)	61	20
Manus	(J)	737	230
New Ireland	(K)	446	139
East New Britain	(L)	216	68
West New Britain	(M)	439	137
North Solomons	(N)	768	240

Table 2. Annual landings (tonnes) at 9 GFPCs in PNG coralline areas, 1981-1985.

Station	1981	1982	1983	1984	1985
Samarai	49	90	209	236	265
Kimbe	57	34	53	66	45
Kupiano	16	12	17	32	30
Lorengau	–	–	16	10	15
Tufi	17	38	53	35	35
Madang	6	13	15	18	20
Lae	14	23	35	40	45
Kavieng	48	34	36	40	40
Kieta	–	–	30	55	60
Total	207	244	464	532	555

Table 3. Catch composition of the Tigak Islands and Port Moresby artisanal reef fisheries.

Family	% of Tigak Islands catch[a]	% of Port Moresby catch[b]
Mugilidae	21.2	4.0
Carangidae	14.0	8.3
Lutjanidae	13.3	4.7
Lethrinidae	10.4	29.3
Serranidae	9.1	2.5
Scaridae	8.1	5.2
Acanthuridae	4.7	6.8
Haemulidae	3.3	3.9
Scomberomoridae	2.7	10.1
Chanidae	2.3	<1.0
Balistidae	1.6	<1.0
Siganidae	1.3	5.5
Albulidae	1.0	<1.0
Hemiramphidae	<1.0	1.1
Belonidae	<1.0	5.2
Gerridae	<1.0	1.7
Mullidae	<1.0	4.5
Other families	7.0	7.2

[a] From Wright and Richards (1985).
[b] From Lock (unpublished data).

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Table 4. Catch per unit of effort (CPUE) for handline, spear, net and troll fishing in PNG waters.

Location	CPUE Handline kg/line/hr	 Spear kg/man/hr	 Net kg/man/hr	 Troll kg/line/hr
Tigak Islands[a]	1.2	2.4	3.6	4.0
Bougainville[b]	1.8	–	–	4.2
Port Moresby[c]	2.5	2.4	2.5	6.9[d]

[a] From Wright and Richards (1985).

[b] From Ito (1983).

[c] From Lock (unpublished data).

[d] Port Moresby troll catch may contain some handline caught fish.

Table 5. Pearl shell and sea cucumber production (in tonnes) from PNG's coralline areas between 1950 and 1984.

Years	Trochus	Green snail	Black lip pearl	Sea cucumber
1950-1954	3,154	869	35	–
1955-1959	2,789	569	61	–
1960-1964	1,406	98	85	27.6
1965-1969	2,024	78	111	42.6
1970-1974	2,326	175	250	24.3
1975-1979	1,323	214	104	15.2
1980-1984	1,279	27	27	27.3

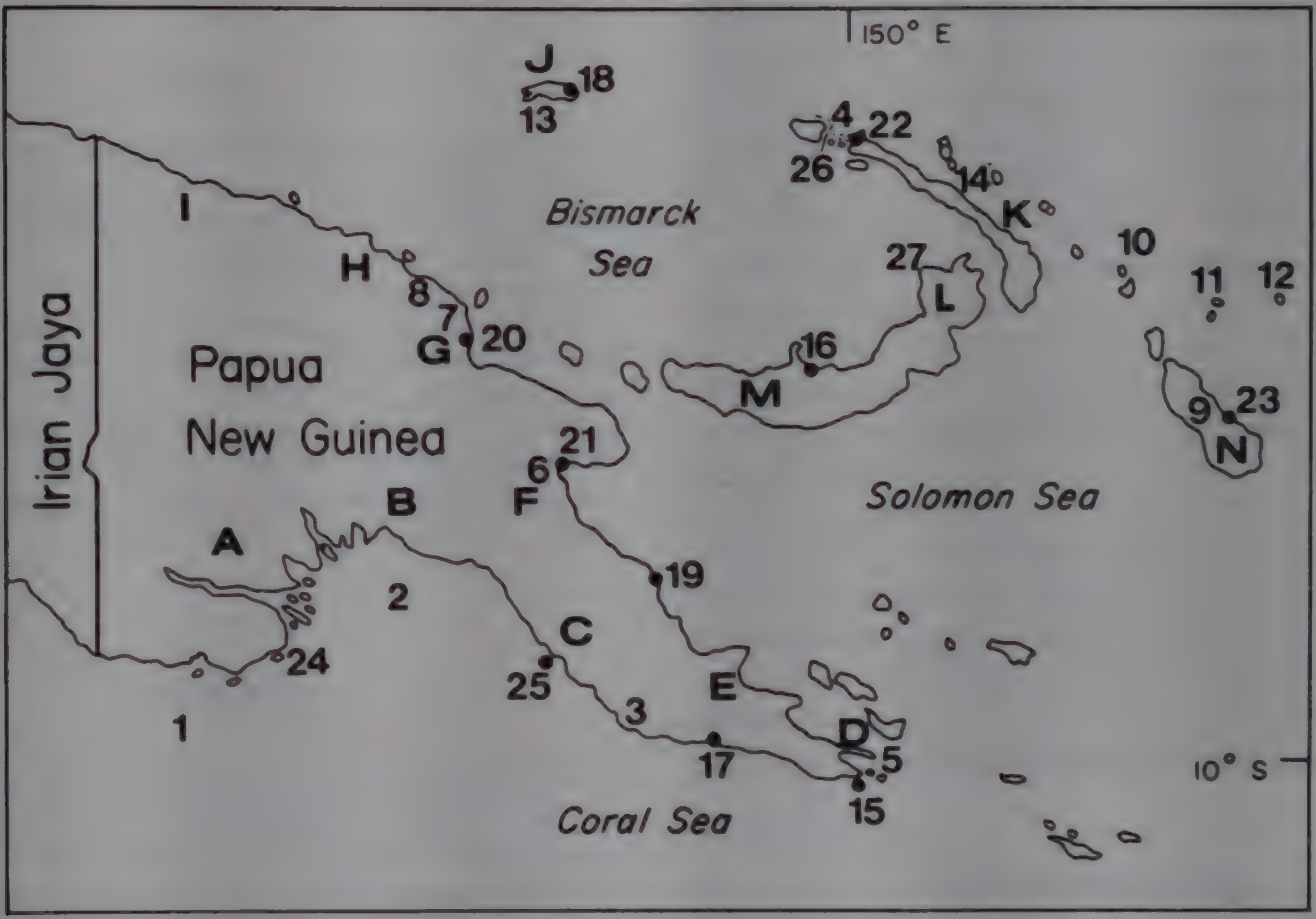
150° E
J
18
13
4
22
26
14
K
Bismarck Sea
I
H
8
7
G
20
10
11
12
27
L
16
M
9
23
N
Irian Jaya
Papua New Guinea
21
6
F
B
A
2
Solomon Sea
19
C
25
24
E
1
3
D
5
17
15
10° S
Coral Sea

Fig. 1. Sketch map of Papua New Guinea showing all places named in text. Letters represent provincial areas listed in Table 1. 1. Torres Strait, 2. Gulf of Papua, 3. Port Moresby, 4. Tigak Islands, 5. Milne Bay, 6. Markham River Mouth, 7. Ramu River Mouth, 8. Sepik River Mouth, 9. North Solomons Province, 10. Nuguria Islands, 11. Cartrets Islands, 12. Mortlocks Islands, 13. Manus Province, 14. New Ireland, 15. Samarai, 16. Kime, 17. Kupiano, 18. Lorengau, 29. Tufi, 20. Madang, 21. Lae, 22. Kavieng, 23. Kieta, 24. Daru, 25. Yule Island, 26. Ysabel Passage and 27. Cape Lambert.

Depth Zonation in a Demersal Fishery in the Samar Sea, Philippines

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Abstract

Analyses of published data from 28 demersal trawl stations in the Samar Sea, Philippines, sampled 11 times in a 16 month-period revealed the existence of two subcommunities divided approximately along the 30-m bathymetric line. TWINSPAN classifications indicated that about half of the dominant species in each subcommunity were depth preferential, while the other half were common to both subcommunities. Although thirteen families of fish contained both ubiquitous and depth preferential species, no families included both deep and shallow preferentials, indicating that the division was not related to competition between sister species. Shallow catches were characterized by *Leiognathus splendens* and *L. equulus*. Deep catches generally included *Saurida undosquamis* and *Nemipterus nematophorus*. *L. bindus* dominated throughout.

Introduction

In classifying communities of soft-bottom fishes in a previously unstudied biogeographical region, it is important to establish whether the results obtained from a given survey are merely transitory, or are meaningful over a reasonable period of time. To investigate this problem, this author conducted exploratory analyses (McManus 1985) on a set of research trawl data published by the College of Fisheries of the University of the Philippines in the Visayas (Armada et al. 1983). The researchers had trawled a set of 28 selected sites during eleven trips to the Samar Sea over a 16-month period. A ban on commercial trawling had gradually been imposed on the fishery over the previous few years, but it is not known how much impact this ban had on the local species composition.

Methods

On the basis of recent discussions and comparisons of available clustering methods (Gauch and Whittaker 1981; Gauch 1982; Greig-Smith 1983; Pielou 1984), the TWINSPAN method of Hill (1979b) was chosen as that most likely to yield a variety of useful information when applied to previously unanalyzed trawl data sets. TWINSPAN incorporates interrelated divisive hierarchical clusterings of sites and species in conjunction with correspondence ordinations, which can yield a table showing the relationships between any set of sites and the distributions of the component species. Whereas many site cluster approaches are difficult to interpret in terms of component species, TWINSPAN results reveal precisely what species distributions are responsible for each site grouping. The divisive approach also has the advantage over aggregative methods that the higher classifications are determined first, thereby avoiding effects of compounded errors on the classifications most likely to be important in generalized interpretations.

Because TWINSPAN is based on divisions of ordination axes, the method can be highly influenced by anomalous samples (outliers) which strongly distort the primary axes on which each division of samples and species is made. Interpretation of the clusterings was facilitated by comparisons with an ordination method which clarified the effects of outliers. Detrended correspondence analysis (Hill 1979a) was selected because of its ability to clearly display the TWINSPAN divisions and its correction of certain nonlinear problems (the arch effect and end point compression - see Pielou 1984).

Results and Discussion

The classification studies revealed that the Samar Sea fishery could be divided into subcommunities above and below the 30-m bathymetric line (Fig. 1). Approximately half of the dominant species in each subcommunity were depth preferential and the other half were ubiquitous with respect to depth (Table 1). Separate analyses of the tables of sites by species from each sampling period revealed a tendency for the primary division to be occasionally displaced by outlying samples, but depth stratification was apparent in all cases. This would suggest that for future analyses of similar communities, classifications based on single sample periods should be treated cautiously, and wherever possible, data from more than one sample period should be compared or combined before analysis to minimize the effects of outliers.

Gradient analyses were performed in the form of individual graphs of the depth distributions of 101 fish species for each sampling period. None of these species changed its status with respect to depth preference during the 16-month study. Sixteen families of fish were represented by two or more species each, including eighty-three species (Table 2). Thirteen of these families included ubiquitous species. None of the families had both shallow and deep preferential species. Thus, while the depth division appears to relate to higher taxonomic classifications, it does not appear to be related to competition between closely related sister species.

An important management implication of the depth stratification concerns the imposition of commercial trawl bans. For at least half of the major species in the fishery, fishing activities below 30-m depth are unlikely to have any major impact on fishing above this transition zone. In order to determine whether or not this holds true for the major ubiquitous species, it would be desirable to determine the nature of the stock boundaries or gradients over depth. Attempts are now underway to determine community structure in other parts of Southeast Asia. If both the stock boundaries and subcommunity boundaries tend to break predictably at some reasonable depth in each region, it may eventually be possible to permit commercial trawling within restricted depth limits without disrupting small-scale fisheries. This would presuppose, however, that an effective means of monitoring and enforcement could be developed beyond those currently available in these regions.

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Table 1. Dominant species and diversity by subcommunity. Percents and cumulative % based on total catches in each site group in kg. Species ranges are ubiquitous (U), shallow (S) or deep (D) as per TWINSPAN classification of mean site by species table. Trends are increasing (+) or neutral (0) in abundance over the 16-month study period as per classification of mean times by species table. Shannon diversities and eveness indices were computed as per Pielou (1977), and are approximations intended only for comparisons between subcommunities. Lower eveness of the deeper community is largely attributable to dominance by *Leiognathus bindus*. *L. splendens* and *L. equulus* characterize shallow subcommunity. Deep stations characteristically include *Saurida undosquamis* and *Nemipterus nematophorus*. *L. bindus* dominates throughout.

	Shallow subcommunity (<40 m)					Deep subcommunity (40+ m)					All stations				
Rank	Taxon	%	cum. %	range	trend	Taxon	%	cum. %	range	trend	Taxon	%	cum. %	range	Trend
1	*Leiognathus bindus*	9	9	U	0	*Leiognathus bindus*	28	28	U	0	*Leiognathus bindus*	22	22	U	0
2	*Leiognathus splendens*	8	17	S	0	*Pentaprion longimanus*	7	35	U	0	*Loligo* spp.	6	27	U	0
3	*Loligo* spp.	7	25	U	0	*Saurida undosquamis*	6	41	D	0	*Pentaprion longimanus*	6	33	U	0
4	*Leiognathus equulus*	6	31	S	0	*Loligo* spp.	5	45	U	0	*Saurida undosquamis*	4	37	D	0
5	*Rastrelliger brachysoma*	4	34	U	0	*Nemipterus nematophorus*	4	49	D	0	*Saurida tumbil*	3	41	U	0
6	*Trichiurus lepturus*	4	38	U	0	*Saurida tumbil*	4	53	U	0	*Upeneus sulphureus*	3	44	U	0
7	*Saurida tumbil*	3	41	U	0	*Upeneus sulphureus*	3	56	U	0	*Nemipterus nematophorus*	3	47	D	0
8	*Apogon* spp.	3	44	U	+	*Decapterus macrosoma*	3	59	U	0	*Leiognathus splendens*	3	49	S	0
9	*Upeneus sulphureus*	3	46	U	0	*Apogon* spp.	3	62	U	+	*Rastrelliger brachysoma*	3	52	U	0
10	*Alepes djedaba*	2	49	S	+	*Fistularia* spp.	2	64	D	+	*Apogon* spp.	3	54	U	+
11	*Pentaprion longimanus*	2	51	U	0	*Priacanthus macracanthus*	2	66	D	+	*Decapterus macrosoma*	2	57	U	0
12	*Selaroides leptolepis*	2	54	S	0	*Sepia* spp.	2	69	U	+	*Sepia* spp.	2	59	U	+
13	*Sepia* spp.	2	56	U	+	*Lagocephalus lunaris*	2	71	U	0	*Trichiurus lepturus*	2	61	U	0
14	*Leiognathus brevirostris*	2	58	S	0	*Rastrelliger brachysoma*	2	73	U	0	*Leiognathus equulus*	2	63	S	0
15	*Stolephorus indicus*	2	60	U	0	*Rastrelliger kanagurta*	2	75	U	0	*Lagocephalus lunaris*	2	65	U	0
16	*Rastrelliger kanagurta*	2	62	U	0	*Priacanthus tayenus*	2	77	U	+	*Rastrelliger kanagurta*	2	67	U	0
17	*Decapterus macrosoma*	2	64	U	0	Triglidae	2	78	D	+	*Fistularia* spp.	2	69	D	+
18	*Stolephorus tri*	2	65	S	0	*Trichiurus lepturus*	1	80	U	0	*Priacanthus tayenus*	2	71	U	+
19	*Lagocephalus lunaris*	2	67	U	0	*Upeneus moluccensis*	1	81	D	0	*Priacanthus macracanthus*	2	72	D	+
20	*Nemipterus japonicus*	2	68	U	0	*Stolephorus indicus*	1	82	U	0	*Stolephorus indicus*	1	74	U	0
	Total taxa	S	159			Total taxa		166			Total taxa		172		
	Total catch (kg)	N	18,400			Total catch		37,100			Total catch		55,500		
	No. tows (1 hr. equiv.)		107			No. tows		201			No. tows		308		
	Catch rate (kg/hr)		172			Catch rate		186			Catch rate		180		
	Shannon index (bels)	H'	3.9			Shannon index		3.3			Shannon index		3.7		
	Eveness	H'/ln (S)	0.77			Eveness		0.66			Eveness		0.71		

Table 2. Subcommunity preferences of species by families. Included are all families with two or more species (excluding rare species). None of the families with shallow preferential species also included deep preferential species. There was no evidence that the depth gradient had been divided because of competition between closely related species.

Family	Shallow	Deep	Ubiquitous	Total
Clupeidae	2		2	4
Engraulidae	2		1	3
Carangidae	9		11	21
Leiognathidae	10		1	11
Lutjanidae	1		1	2
Gerridae	5		1	6
Pomadasyidae	2		2	4
Scombridae	1		3	4
Teraponidae	2			2
Scaenidae	3			3
Synodontidae		1	2	3
Serranidae		3	1	4
Priacanthidae		1	1	2
Nemipteridae		1	4	5
Mullidae		2	3	5
Sphyraenidae			4	4

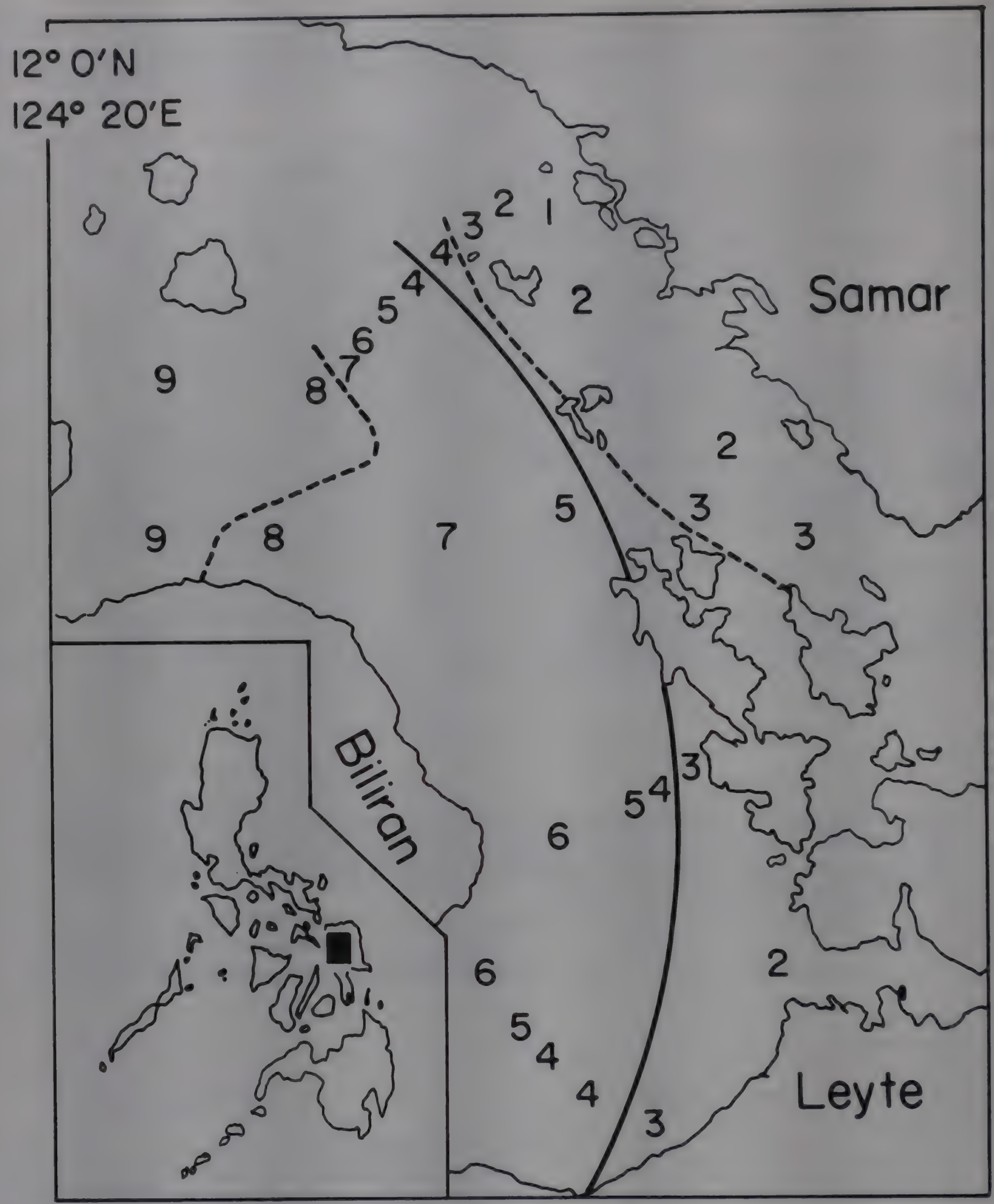

Fig. 1. Map of study area indicating subcommunities of demersal fishes based on 172 taxa. Each number represents the lower depth limit of a trawl site (x 10 m) and its approximate location. Sites were divided by TWINSPAN analysis based on mean weights of species at each site. The primary division among the sites is indicated by the thick solid line. This division was arrived at naively with respect to depth, but coincides geographically with the 30 to 40 m bathymetric line. Dashed lines indicate secondary divisions within each primary subcommunity. Analyses of the sites by species table from each sampling time indicate that the division into deep and shallow subcommunities is relatively constant, while further subdivisions are unstable and geographically inconsistent. Map modified from Armada et al. 1983.

Survey of Coral Reefs, Iligan Bay, Philippines

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Abstract

A project was started in 1983 with the primary objective of assessing the formation, distribution and systematics of corals and determining the utilization of the coral reefs of Iligan Bay, Philippines. Seven stations were established and monitored. The west coast of the Bay is characterized by pseudobarrier reef types, the rest by fringing reefs. Species composition varied considerably from one station to another. However, live coral formations generally concentrated along the reef slopes, becoming lesser towards reef flats closer to the shoreline. This could be due to excessive exposure during low tides and to some degree to natural coastal siltation. A total of 204 species of reef-forming corals belonging to 46 genera were collected and classified. Massive *Porites* ranked first in dominance followed by ramose *Porites*, ramose *Acropora*, massive *Favia* and *Galaxea*. Fifteen species of shellfish were identified to be of commercial importance. Five species of echinoderms and three species of algae were also identified to be of economic value to local reef fishermen. Twenty fishing gears used in reef areas were identified and classified. Observed perturbations from the sampling area included coral and shell collection, blast fishing, fishing with cyanide and rotenone, anchor damage and *Acanthaster plancii* infestation. However, a major cause of coral destruction may be the heavy siltation resulting from excessive deforestation, agricultural malpractice, quarrying and land-based structural development.

Introduction

The Philippine Islands are rich in coral reef resources, but most are showing evidence of degradation under stress from natural catastrophes and man-induced interference (Montecillo 1980). This led the government to enforce a ban (Presidential Decree 1219) on the gathering and exportation of ordinary or stony corals. There is a need for the adoption of effective conservation measures as well as a search for measures of wiser management of their high productivity (NMRC 1979). Conservation, on the other hand, requires a nationwide assessment of the current coral reef status (Gomez et al. 1981).

This research project was primarily aimed to assess the status of the coral reefs in Iligan Bay. Secondary objectives were to assess the distribution, systematics and status of corals in the study areas; to determine the existing coral reef utilization and fishery; and to reevaluate the status of corals with ecological parameters and other coral reef-related marine activities.

Methods

The project started in February 1983 with the survey of probable sampling stations based on maps and navigational charts. Seven stations in Iligan Bay were established. The transect-quadrat method was used and data were gathered by skin diving and SCUBA diving. The transect was calibrated using 5-mm nylon rope stretched from the shoreline to the reef edge. Quadrat samplings for every 5-m interval were limited to points where coral growth started and ended on areas where corals were almost zero and where depth limited sampling activities. Samplings for every station were done for three days every month for three months. Corals and other distinguishable flora and animal associates were quantified using a 1 x 1 m brass quadrat, which was divided into twenty-five 20 x 20 cm subquadrats.

The data gathered were: percentage of live and dead coral; percentage of live coral per genera and per growth forms; number of colonies; plants and animal associates; substratum types; and identification of corals near the transect line not covered by the quadrat. For species not identified, collections were made. Quantification of the number of colonies for species growing extensively like *Acropora* and huge *Porites*, was done by modified counting (Scheer 1978). Environmental parameters were also monitored. Data on reef fishery and utilization were gathered through field and market survey and interview.

Statistical analyses of the species composition and distribution of coral species followed the formulae of Odum (1971) and Shannon and Weaver (1974). The degree of abundance was determined by using importance values (IV) computed for each coral genera per growth form and the formula adapted from Randall et al. (1975).

Results and Discussion

The survey established that the coral reefs of Iligan Bay are of fringing types except for a few pseudobarrier types formed by islets with wide reef flats separated by deep channels in the southwestern coast of the Bay.

Fig. 1 shows a general reef profile of the seven stations. Reef areas with wide reef flat tend to follow a uniform trend. The shoreline is bordered by sandy muddy substratum followed by a wide stretch of sandy rocky and rocky substrate.

Coral colonies growing extensively in herds or beds along the reef sites of the Bay are plotted in Fig. 2. Arborescent or ramose type corals represented by genera *Acropora* and *Porites* were the most active growing corals. However, Station 3 showed massive *Euphyllia fimbriata* growing in large herds extending over an area of about 1,000 m^2. Station 5, located within the industrial portion of the Bay, had large thickets of very fragile and slim *Anacropora puertogalerae*.

Based on computed IV, massive *Porites* ranked first in dominance with IV of 373.37. This was followed by *Porites* ramose, 216.61; *Acropora* ramose, 143-69; *Favia* 125.56; and *Galaxea,* 116.24. While massive and ramose *Porites* were very prominent in almost all stations, the branching *Acropora* and massive *Euphyllia* were dominant only in Stations 2, 3 and 4. Massive *Favia, Favites* and *Galaxea* were evenly distributed in all stations.

Table 1 shows the summary of estimates of live and dead coral cover. Four stations showed fair live coral cover and three were poor compared to the results of Gomez et al. (1981).

Stations 1, 2 and 3 showed signs of *Acanthaster plancii* infestations. Station 2 had a density of 43.3/1,000 m^2 while Stations 1 and 3 had an average density of 15.6 and 27.5/1,000 m^2, respectively. Endean (1977) indicated that a mean density of 14 adult *A. plancii* per 1,000 m^2 is sufficient to kill hard cover of an average reef.

Within the coralline area, several species of algae, sponges and polychaetes were recorded. *Halimeda opuntia, Gelidium* sp. and blue-green algae were observed to be the most common associates of corals growing primarily on dead corals.

In Table 2, the ranges of physicochemical parameters of the seven sampling stations are shown. Water temperatures and salinities were almost uniform. Salinities were within the limiting range for coral growth based on the works of Nybakken (1982). Turbidity readings were not consistent with live coral status.

A coral collection totalled 1,231 specimens. Laboratory identification of growth forms, calice sizes, septal structures and other features revealed 204 species belonging to 46 genera.

Table 3 shows a list of fishing gears operated in the reef areas in Iligan Bay. Popular among the reef fishermen were spear fishing, hook and line and fish traps. Due to low income of fishermen, some shifted to a more efficient but destructive method of fishing, such as to a gear called *bahan-bahan* or *lamba-lamba* (modified set gill net) in Station 6. This type of gear is similar to *muro-ami* as reported by Carpenter and Alcala (1977) in which a vertical scare line with stone weight is used. The weight damages coral.

Illegal methods, like blast fishing, were common in Stations 2, 3 and 6. From interviews, the average number of illegal fishing operations was about three blasts a day. This was confirmed by several observations in the area.

Coral quarrying was observed in Stations 2, 3 and 6. The coral is used as construction and filling material.

Discussion

The corals of Iligan Bay showed a relatively poor to fair condition. This may be due partly to manmade and natural interferences, such as unlimited coral and shell collections, blast fishing, anchor damage, effluents from industrial plants and *A. plancii* infestation. However, there were strong indications that natural siltation brought by river tributaries around the Bay contributed to the condition of the corals. Filtration is accelerated by deforestation, agriculture malpractices, quarrying and land-based structural developments. It is recommended that a quantitative study and evaluation of some active environmental parameters suspected to have adverse effects on coral growth and ecological balance be conducted in the near future. Information on the quantity of exploitable reef resources is wanting. A quantitative assessment is recommended. Furthermore, the national government should seriously enforce the conservation laws affecting the natural resources, specifically the renewable coastal resources.

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Table 1. Summary of data for the seven stations surveyed along Iligan Bay from January 1983 to December 1984.

	Station						
	I	II	III	IV	V	VI	VII
Live coral cover (%)	10.10	27.24	33.53	37.43	20.02	20.70	18.81
Dead coral cover (%)	38.39	39.76	30.63	21.50	48.20	51.70	59.32
Sandy/loose substrate cover (%)	32.61	33.03	35.82	41.07	31.98	27.58	21.86
Total number of genera	8	37	39	31	33	29	27
Total number of species collected	46	66	47	79	54	60	61
Total number of quadrats sampled	16	102	102	72	82	72	79
Total number of transects	2	3	3	4	4	3	2
Density (colonies/m^2)	3.3	4.23	7.83	10.37	8.84	–	–
$\bar{H}_c$	0.93	1.06	1.08	1.17	0.78	0.88	1.11
SD	1.06	1.87	2.00	1.93	3.11	2.46	1.01
$\bar{X}$	0.77	0.73	0.90	1.12	1.11	0.85	0.89

Table 2. Range of physicochemical parameters recorded in the seven stations along Iligan Bay from June 1983 to May 1984.

Station	Salinity (ppt)	Turbidity (Secchi disk)	Water temperature (°C)
I (Sulawan)	32.5 – 34.0	3 – 19	29.5 – 32.0
II (Samburon)	31.5 – 36.0	5 – 17	26.5 – 31.0
III (Oroquieta)	31.0 – 35.0	3 – 20	28.8 – 31.0
IV (Lampason)	32.0 – 36.5	11 – 22	25.5 – 28.0
V (Tag-ibo)	31.0 – 35.8	5.8 – 15	28.5 – 31.0
VI (Jimenez)	35.0 – 39.0	6 – 12	27.0 – 29.7
VII (Kauswagan)	33.0 – 38.0	8 – 16	29.0 – 30.5

Table 3. List of identified fishing gears used in the coral reefs of Iligan Bay.

1. Bubo (large basket-type fish trap)
2. Panggal (medium basket size fish trap)
3. Timing (small basket type fish trap)
4. Sanggab (fish trap)
5. Sibot (scoop net)
6. Pana (spear)
7. Ganso (spear with bait)
8. Pasol (hook and line)
9. Baling/Hulbot tila (beach seine net)
10. Bungsod (fish corral)
11. Sabay (one-man encircling gill net)
12. Pukot (bottom set gill net)
13. New look (stationary lift net)
14. Bahan-bahan (drive-in net, medium)
15. Lamba-lamba (set gill net)
16. Laya (cast net)
17. Pangtamban (bottom gill net)
18. Tapsay (drive-in net, small)
19. Tiro (blast fishing)
20. Tubli (rotenone)

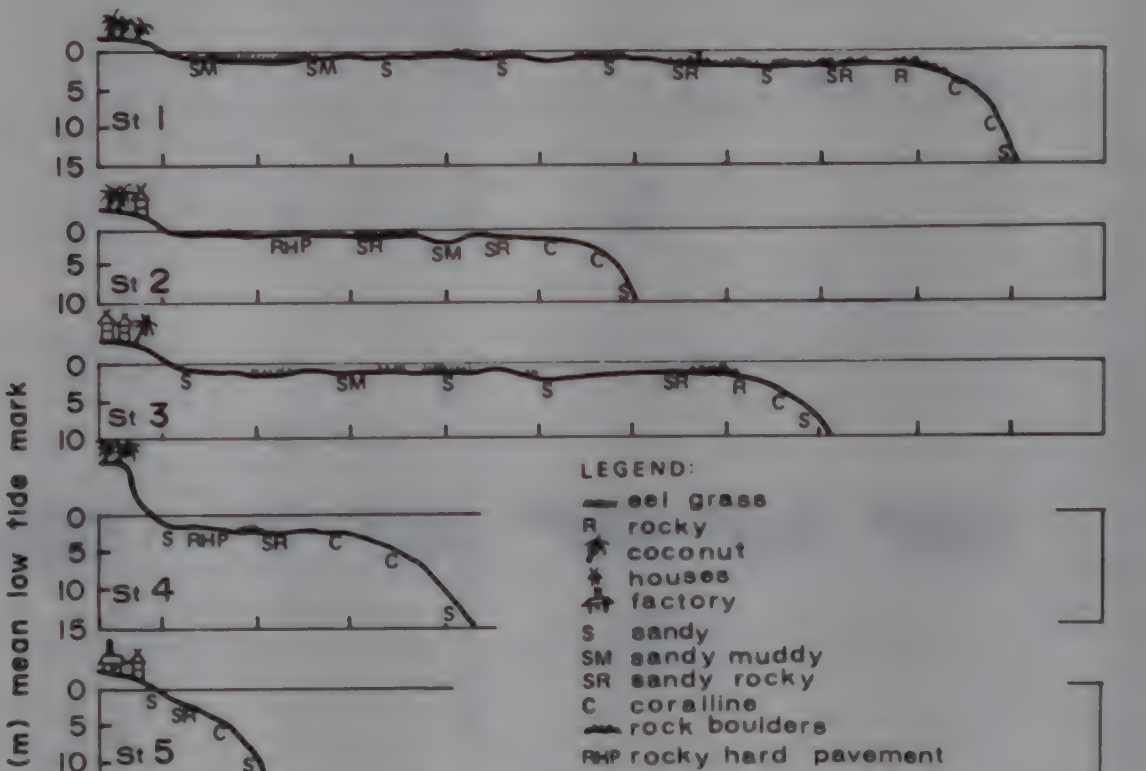

Fig. 1. Schematic diagram showing depth profile and substrate type of stations along Iligan Bay.

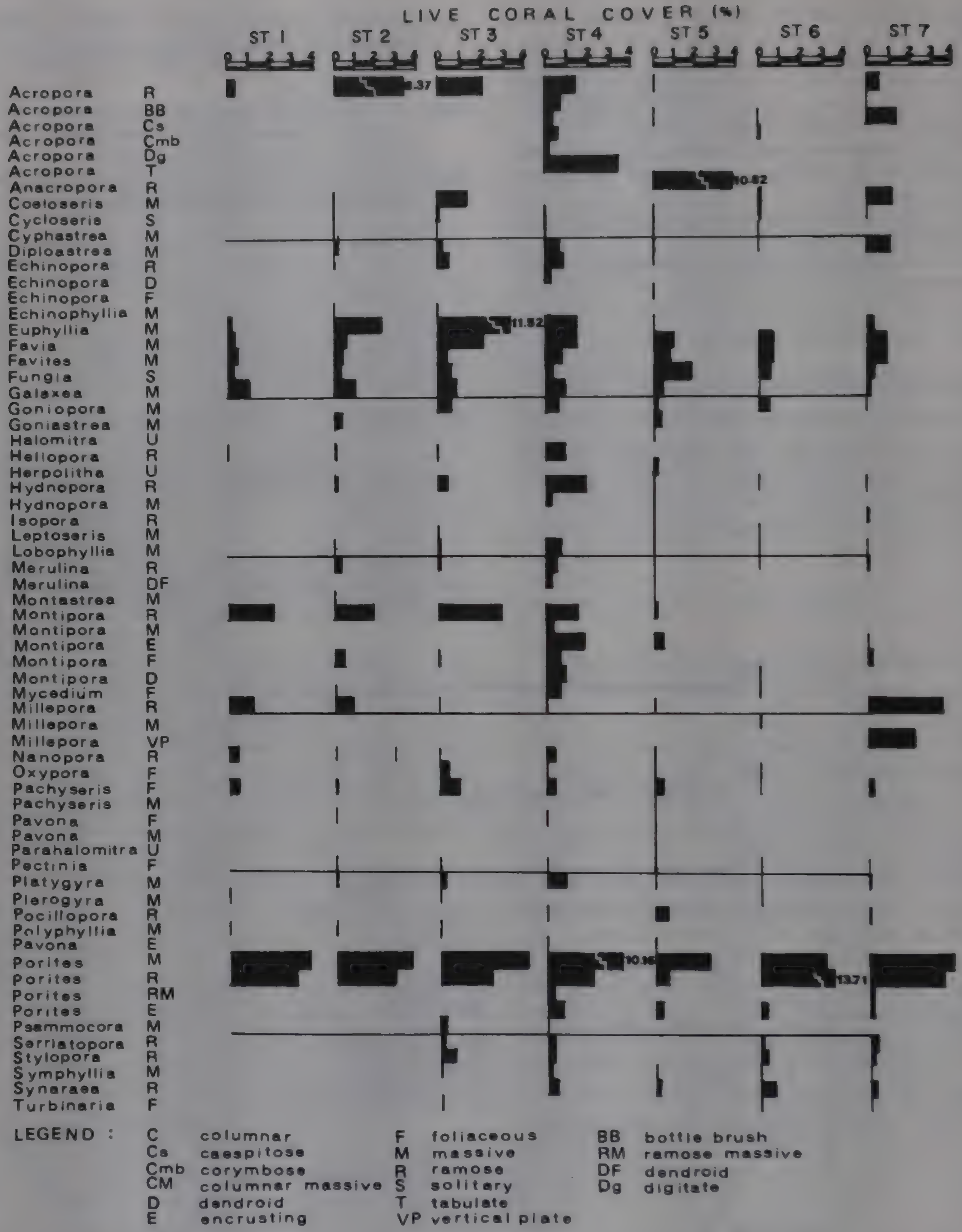

Fig. 2. Bargraph showing distribution of coral colonies among stations established along Iligan Bay from Jan. 1983 to Dec. 1984.

Some Practical Extensions to Beverton and Holt's Relative Yield-Per-Recruit Model*

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Abstract

The relative yield-per-recruit (Y'/R) model of Beverton and Holt estimates Y'/R values based on few inputs, i.e., c = mean length at first capture/asymptotic length, ratio of natural mortality to growth (M/K) and exploitation rate (E = F/Z). However, when used in conjunction with high M/K values and/or a wide selection range, which frequently occur in small, short-lived tropical fishes and invertebrates, the model fails to estimate the optimum level of E for a given fishery. A number of approaches are presented to correct for this and related deficiencies.

Introduction

Following the development of an age-structured theory of fishing by Beverton and Holt (1957), these authors subsequently developed (Beverton and Holt 1964) a length-structured version of their yield-per-recruit model ideally suited for use in data-sparse, tropical setups.

The length-structured yield-per-recruit model of Beverton and Holt (1964) had only three variables (c, M/K and E: see below for definitions), against seven in the 1957 model (W_∞, K, t_o, t_c, t_{max}, M, F; see below), the gap between the two models being bridged by a set of assumptions that seemed reasonable enough, and lots of serious algebra.

Since its publication, the length-structured, "relative" yield-per-recruit model has been applied widely, notably in the tropics, and formed the base for a number of useful generalizations in fishery management (see e.g., Gulland 1971; Sinoda et al. 1979; Pauly 1984).

Sometimes, unwary users have interpreted results in ways not intended by the authors, the most common misinterpretation being that predicted yields *per recruit* (Y/R) are perceived as *yield* predictions (Y), without regard to recruitment. Also the decline in catch per effort concomitant with increased yield per recruit is often not considered.

In this paper, a number of concepts and equations are presented which help in interpreting results obtained using Beverton and Holt's length-structured yield-per-recruit model. These concepts and equations parallel those developed for use with the age-structured version(s) of yield-per-recruit models such as

$$\frac{Y}{R} = F \cdot e^{-Mr_2} W_\infty \cdot \left[\frac{1 - e^{-Zr_3}}{Z} - \frac{3e^{-Kr_1}(1 - e^{-(Z+K)r_3})}{Z+K} + \frac{3e^{-2Kr_1}(1 - e^{-(Z+2K)r_3})}{Z+2K} - \frac{e^{-3Kr_1}(1 - e^{-(Z+K)r_3})}{Z+3K} \right] \quad \ldots 1)$$

where $Z = F + M$, $r1 = t_c - t_o$, $r2 = t_r - t_o$ and $r3 = t_{max} - t_c$.

The model assumes isometric von Bertalanffy growth, natural, fishing and total mortality rates (M, F and Z, respectively) expressed by negative exponential curves and also assumes that all fish of a given cohort enter the fishing ground or become catchable by the gear or leave the fishery at the same ages through "knife-edge" recruitment (t_r), selection (t_c) and "derecruitment" (t_{max}), respectively. These latter assumptions are reasonable with long-lived fishes, in which the biomass above t_r or t_c forms the overwhelming part of stock biomass, but not necessarily with small animals, in which, e.g., the selection range may span the entire size distribution (see below). De-recruitment, on the other hand, can usually be ignored (especially when Z is high), by setting $t_{max} = \infty$ nd simplifying equation (1) to

$$\frac{Y}{R} = F \cdot e^{-Mr_2} W_\infty \cdot \left[\frac{1}{Z} - \frac{3e^{-Kr_1}}{Z+K} + \frac{3e^{-2Kr_1}}{Z+2K} - \frac{e^{-3Kr_1}}{Z+3K} \right] \quad \ldots 2)$$

(Jones 1957)

From this, assuming an isometric length-weight relationship, Beverton and Holt (1964) derived the length-structured, "relative" yield-per-recruit model.

$$\frac{Y'}{R} = E(1-c)^{M/K} \cdot \left[1 - \frac{3(1-c)}{1 + \frac{(1-E)}{(M/K)}} + \frac{3(1-c)^2}{1 + \frac{2(1-E)}{(M/K)}} - \frac{(1-c)^3}{1 + \frac{3(1-E)}{(M/K)}}\right] \quad \ldots 3)$$

in which Y'/R is the relative yield per recruit, E = F/Z, $c = L_c/L_\infty$, where L_c is the length corresponding to t_c, where t_r is set at zero, and where L_∞ is the asymptotic length, corresponding to W_∞ in equation (1) and (2). The relationship between Y/R as expressed by equation (2) and relative yield per recruit as expressed by equation (3) is given by

$$Y/R = (Y'/R) \cdot W_\infty \cdot \exp - (M(t_r - t_o)) \quad \ldots 4)$$

Model Extension I: Derivation of a Biomass Per Recruit Index

Because unwary model users equate yield per recruit and yield, and also in order to predict relative catch per effort, biomass-per-recruit curves are often drawn as a function of F along with yield-per-recruit curves. Such biomass curves can be derived by dividing equation (2) by F.

Tables with computed relative equivalents of B/R (i.e., B'/R) are given in Beverton and Holt (1964). Since the equation they used was not given explicitly, an appropriate equation is presented here, i.e.,

$$\frac{B'}{R} = \frac{(1-E)\left[1 - \frac{3(1-c)}{1 + \frac{(1-E)}{(M/K)}} + \frac{3(1-c)^2}{1 + \frac{2(1-E)}{(M/K)}} - \frac{(1-c)^3}{1 + \frac{3(1-E)}{(M/K)}}\right]}{\left[1 - \frac{3(1-c)}{1 + \frac{1}{(M/K)}} + \frac{3(1-c)^2}{1 + \frac{2}{(M/K)}} - \frac{(1-c)^3}{1 + \frac{3}{(M/K)}}\right]} \quad \ldots 5)$$

which has the useful property of being equal to unity when E = 0 and equal to 0 when E = 1. Conversion of this biomass index to absolute biomass per recruit can be performed, in analogy to equation (4) by the relationship

$$B/R = (B'/R) \cdot W_\infty \cdot \exp - (M(t_r - t_o)) \quad \ldots 6)$$

Note that MSY is generated when relative biomass is 50% or 37% of virgin stock, at least in terms of the Schaefer (1957) and Fox (1970) models, respectively.

Model Extension II: $E_{0.1}$ Concept Analogous to $F_{0.1}$

Identification, in the context of rapid assessment, of the values of F generating Maximum *Sustainable* Yield ("F_{opt}") is not easy, and practitioners have therefore developed a number of rules of thumb, the most used of which is $F_{opt} = 0.5$. Another definition is $F_{opt} = F_{0.1}$, the latter term being defined as the fishing mortality at which the marginal increase of yield per recruit is 1/10 of its value at F = 0 (Gulland and Boerema 1973). Since the first of these rules of thumb has been shown to overestimate F_{opt} (Beddington and Cooke 1983), an equation is given below which allows estimation of $E_{0.1}$, the exploitation rate at which the marginal increase of relative yield per recruit is 1/10 of its value at E = O.

The first derivative of equation (3) is

$$\frac{d(Y'/R)}{dE} = [(1-c)^{M/K} \cdot (Y'/R)] + [\frac{3E(1-c)^{1+M/K}}{M/K} \cdot A] \quad \ldots 7)$$

where

$$A = 2(1-c) \cdot \left(1 + \frac{2(1-E)}{M/K}\right)^{-2} - (1-c)^2 \cdot \left(1 + \frac{3(1-E)}{M/K}\right)^{-2} - \left(1 + \frac{1-E}{M/K}\right)^{-2} \quad \ldots 8)$$

which can be solved for any value of E, including E = 0 and E = 1. Using equation (7) it is simple matter to identify using an appropriate search algorithm, for a given pair of M/K and c values, the value of E generating a value of d(Y'/R)/dE equal to 1/10 of the value of d(Y'/R)/dE at E = 0. Equation (7), obviously, can also be used to estimate the value of E at which yield per recruit is maximized, i.e., the value of E at which d(Y'/R)/dE is equal to zero.

Model Extension III: Relationship Between Exploitation Rate and Mean Length of the Fish in the stock

Beverton and Holt (1956) showed that, given the same assumptions as those used in the derivation of the yield-per-recruit model,

$$Z/K = (L_\infty - \bar{L}) / (\bar{L} - L') \qquad \ldots 8)$$

where $\bar{L}$ is the mean length computed from L' upward, the latter being a length "not smaller than the smallest length of fish fully represented in catch samples." Note that $L_c < L'$, except in the case of knife-edge selection, where $L_c = L'$.

Equation (8) can be solved for L (J. Hoenig, pers. comm.), in which case we have

$$\bar{L} = (L_\infty + (L' \bullet Z/K)) / ((Z/K) + 1) \qquad \ldots 9)$$

Now since E = F/(F+M) and E = 1 - (M/K)/(Z/K), we also have

$$\bar{L} = [L_\infty + (\frac{M/K}{1-E} \bullet L')] / [((M/K)/(1-E)) + 1] \qquad \ldots 10)$$

for $0 \leq E < 1$. Thus, one can, when performing a relative yield-per-recruit assessment, also assess straightforwardly the reduction of mean length brought about by an increase of E.

Model Extension IV: Compensating for the Effects of a Wide Selection Range

In large, long-lived fish such as cod or plaice, the selection process usually takes place over a relatively narrow range of sizes, such that the assumption of "knife-edge" selection is acceptable. In some small animals such as shrimps caught by trawls, the selection range may cover most size classes represented in the population. In such cases, yield-per-recruit computations involving the assumption of knife-edge selection may involve a large bias. A simple method is developed here to show the extent of and to help overcome this bias.

Selection curves provide a probability of capture (P_i) for catch-length class (i) between L_{min}, the smallest, and L_{max}, the maximum length represented in the available catch samples. In the unmodified model, it is assumed that $P_i = 0$ when $L < L_c$ and $P_i = 1$ when $L > L_c$ (hence also $L' = L_c$). The implicit assumption here is that, if selection is not knife-edged, the yield from the fish caught below L_c will compensate for the yield losses due to the fact that not all fish larger than L_c are caught.

Although some compensation may occur, the assumption of knife-edge selection does generate a large bias, especially for high values of E, as can be shown by reformulating Beverton and Holt's method for computation of yield per recruit for different E values over the lifespan of a fish (section "f" in Beverton and Holt 1964) such that E is assumed constant, but P variable. This gives

$$Y'/R = \sum_{i=L_{min}}^{L_\infty} P_i ((Y'/R)_i \bullet G_{i-1}) - ((Y'/R)_{i+1} \bullet G_i)) \qquad \ldots 11)$$

in which $(Y'/R)_i$ and $(Y'/R)_{i+1}$ refer to relative yield per recruit as computed from the lower limit of length class (i), P_i refers to the probability of capture between L_i and L_{i+1} (see Table 1), while G_1 is defined by

$$G_i = \prod_{j=1}^{i} r_j \qquad \ldots 12)$$

and where r_i is a factor expressing the proportion of recruits of length L_i which survive, grow and reach length L_{i+1}, which is computed, for $0 < E < 1$, from

$$r_i = \frac{(1-c_i)^{(M/K)(E/(1-E))P_i}}{(1-c_{i-1})^{(M/K)(E/(1-E))P_i}}; \; r_{L_{min-1}} = 1; \; r_{L_\infty} = 0 \qquad \ldots 13)$$

which is analogous to Beverton and Holt's "reduction factor", but considers P as a variable and has the exponent (M/K)(E/(1-E)) instead of F/K.

Replacing the (Y'/R) terms in equation (11) by (B'/R) as given by equation (5) is straightforward and will lead to estimates of biomass per recruit independent of the knife-edge assumption.

Table 1 gives probabilities of capture for selection gives with increasingly large ranges, from knife-edge selection in case 1 to a selection range spanning most of the range between 0 and L_∞ in case 3. In Fig. 1A, departure from knife-edge selection has a profound impact on yield-per-recruit estimation, particularly at high values of E; similar results are obtained for relative biomass per recruit (Fig. 1B).

Model Extension V: and Empirical Equation to Predict M/K

Beverton and Holt (1964) presented their relative yield-per-recruit model in the form of yield tables at a time when microcomputers and programmable calculators did not exist. That they were able to include in their table a realistic range of M/K values is due to a previous review of the growth and mortality of fish (Beverton and Holt 1959), in which they showed that M/K varies less between stocks than either K or M alone. Pauly (1980) used their data and a number of other data sets to demonstrate the existence in fish of strong partial correlations between M on the one hand and L_∞ and mean environmental temperature on the other. Here, the data compiled in Pauly (1980) (see Pauly 1985 for correction of 4 outliers), were used to show that M/K in fishes is affected by the temperatures of their habitat, i.e.,

$$\log_e (M/K) = -0.22 + 0.30 \log_e T \qquad \ldots 14)$$

where T is the mean water temperature, in ^{o}C ($r = 0.308$, 173 d.f., $P < 0.001$; s.e. of slope = 0.07; range 3-30^{o}C).

Equation (14) implies that the values of M/K commonly used for stock assessment in temperate waters (say 10^{o}) should be used in the tropics (say 30^{o}) only after multiplication by a factor of about 1.4.

Model Extension VI: An Alternative for Plotting Yield Isopleth Diagrams for High Values of M/K

In the tropics, and/or when dealing with small animals, use of the relative yield-per-recruit model often implies use of high M/K values (<2 see above).

In such cases, however, yield per recruit have maxima occurring at values of E (> 0.5) and often corresponding to extremely high values of fishing mortality. When L_c/L_∞ is near 0.5, yield isopleth diagrams based on equation (3) then usually consist of 4 quadrants (A-D) with properties as given in Table 2.

Discussion

The various extensions of the length structured yield-per-recruit model presented here should help make its applications to tropical fish and invertebrates considerably easier, and lead to results that will be less biased and straightforward to interpret. These extensions also illustrate how "classical models" rather than being rejected out of hand can be adapted to better fit situations for which they may not have been originally intended.

The listing of a BASIC program implementing the equations presented in this paper is available on request from the authors.

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Table 1. Probabilities of capture for a simulation of effects on relative yield per recruit of increasing the selection range (for $L_\infty = 10$, $M/K = 2$).

Length class	1.0-1.9	2.0-2.9	3.0-3.9	4.0-4.9	5.0-5.9	6.0-6.9	7.0-7.9	8.0-8.9
Case 1[a]	0	0	0	0	1	1	1	1
Case 2	0	0	0.1	0.3	0.7	0.9	1	1
Case 3	0	0.1	0.2	0.4	0.6	0.8	0.9	1

[a] Knife-edge selection.

Table 2. Properties of the 4 quadrants of relative isopleth diagrams, when $M/K > 2$, critical size ratio $(L_c/L_\infty) = 0.5$ and critical exploitation rate $E = 0.5$.

Quadrant location	Fishing regime[a]	Assessment	Possible interventions[b]
A $c = 0.5$ to 1 $E = 0$ to 0.5	large fish are caught at low effort levels	underfishing	let effort increase strongly or do nothing[c]
B $c = 0$ to 0.5 $E = 0$ to 0.5	small fish are caught at low effort levels	eumetric fishing developing fishery	do nothing, but note that mesh sizes will have to be increased as effort increases
C $c = 0$ to 0.5 $E = 0.5$ to 1	large fish are caught at high effort level	eumetric fishing developed fishery	effort must be stabilized and possibly reduced[b]
D $c = 0$ to 0.5 $E = 0.5$ to 1	small fish are caught at high effort levels	overfishing	increase mesh size and decrease effort

[a] In terms of yield per recruit only.

[b] Considering that open-access fisheries will become overcapitalized if not probably managed.

[c] Because the fishery is probably generating maximum economic yield.

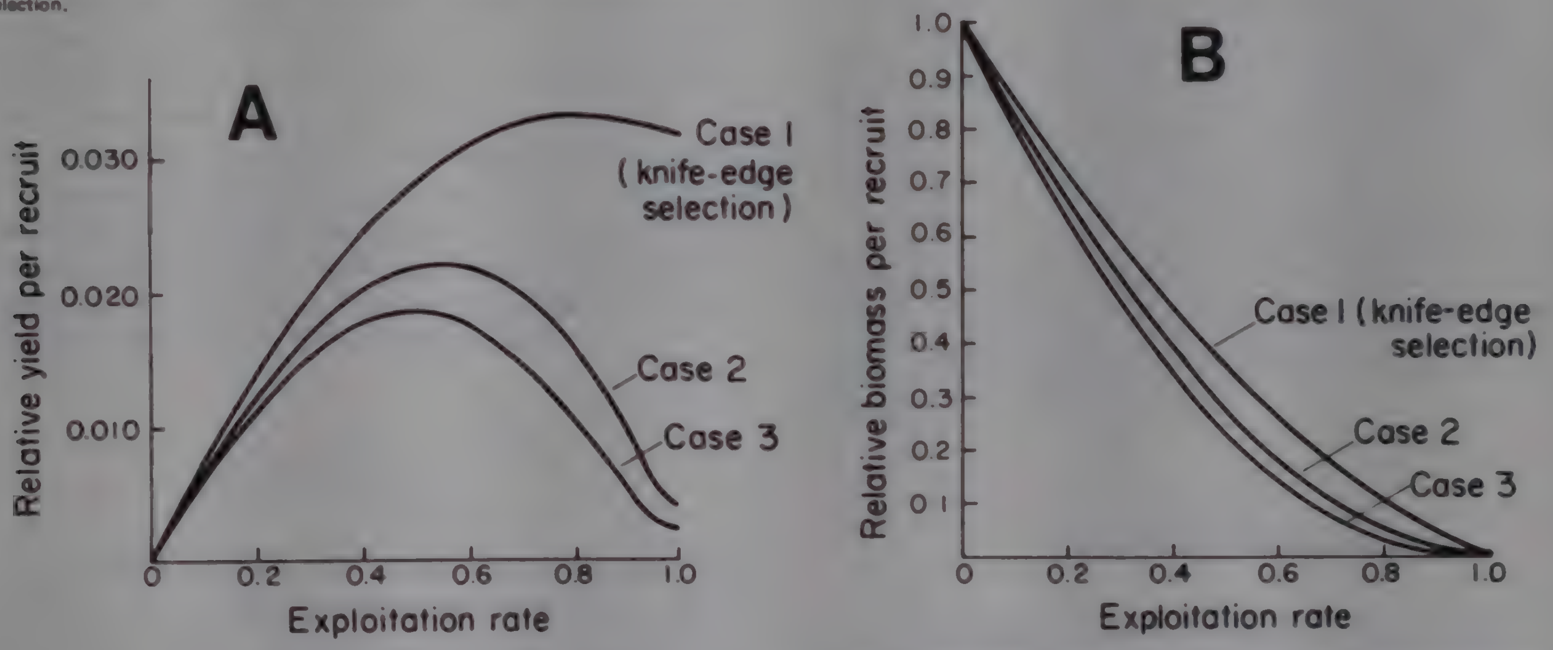

Fig. 1. Effect of an increasingly wide selection range on relative yield per recruit (A) and relative biomass per recruit (B) as assessed through application of equation (11) to the data in Table 1. Note that the knife-edge assumption leads to overestimates of yields and of optimum effort; based on Table 1.

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Reef Fish Yields and Nonreef Catch of Pamilacan Island, Bohol, Philippines

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SAVINA, G.C. and A.T. WHITE. 1986. Reef fish yields and nonreef catch of Pamilacan Island, Bohol, Philippines, p. 497-500. *In* J.L. Maclean, L.B. Dizon and L.V. Hosillos (eds.) The First Asian Fisheries Forum. Asian Fisheries Society, Manila, Philippines.

Abstract

Data on daily fish catches from the 1.8-km^2 coral reef and surrounding deepwater of Pamilacan Island, Bohol, were recorded by fishermen-volunteers living on the island during the study period 1 April 1985-31 March 1986. Reef fish yield including gleaning of 17.9 t/km^2/year was higher than expected given the inferior quality of the coral reef as determined by line transects and snorkeling surveys. High nonreef catches totalling 26 t/year, compared to 19.3 t/year of finfish from the reef, reflect the fishermen's preference for large pelagic fishes, e.g., manta and sting rays, sharks, mackerel and tuna. Seventy-four per cent of the island's income came from the nonreef catch.

Introduction

Recent reviews of fish yields from coral reefs (see Marten and Polovina 1981; Munro and Williams 1985; Alcala and Gomez 1985) report harvests ranging from 1 t/km^2/year to 30 t/km^2/year. Munro (1984) suggests that "a figure for demersal and neritic pelagic fish production of 4-6 t/km^2 of *coralline shelf* seems to be valid as a generalization of what can be taken by moderately heavy exploitation" and that the maximum harvests may be in the 10-20 t/km^2 range. He goes on to discuss the findings of Wass (1982) in Samoa, where an average 26 t/km^2/year of fish and invertebrates was taken on intensively exploited shallow reefs (to 8-m isobath).

The "productivity" of coral reefs depends on which organisms one decides to count, as well as on the topography of the reef, its substrate and depth, the methods used to fish it and the fishing intensity. In this paper yields from fishing and gleaning are presented for Pamilacan Island, a coralline island in the central Philippines. There are about 180 households on the island and an estimated 180 fishermen. Although some farming is done, the main source of livelihood is the sea.

Methods

Local fishermen-volunteers compiled daily lists of fish catches, gear and price per kg from April 1985 to March 1986. The number of gleaners present on the reef was counted during each low tide.

Ten fishing families provided prospective data on yields per unit effort, average earnings and disposition of fish catch. General demographic data and data on the number of fishing boats and other gear were obtained through baseline survey interviews with 50% of the island household heads.

Coral reef substrate data were gathered along 60-m line transects randomly placed on the gradual and steep slope habitats (White 1984). Six transects were made, three in each habitat type. A check on this substrate data was obtained by snorkeling surveys.

Fish diversity and abundance were measured by counting species and numbers within a 750-m^2 area (Russ 1984). Fifteen replicates were done, and those fish counted were limited to a preselected list of about 120 common and important species.

Environmental Features

Pamilacan Island is a low (50-m) coralline island less than 200 ha in area. Pamilacan's 180 ha fringing coral reef (1.80 km^2 to the 20-m isobath) is mostly flat and gradually sloping. Soft coral accounts for 12% of the substrate cover, hard coral for less than 5%, while rubble (23%) from disturbances such as dynamiting, blocks (23%) and sand (32%) dominate. This substrate pattern contrasts dramatically to that of Apo Island, another small Visayan island, which displays a coral cover in excess of 64% (32% hard coral). Butterflyfish species richness and abundance, indicators of coral quality, are both low (34 individuals/750 m^2) at Pamilacan compared to Apo (120/750 m^2).

Systematic fish counts showed an average of 34 species and 1,353 individuals/750 m^2 (Table 1), figures not significantly different from those for Apo Island. The most abundant food fishes were fusiliers, surgeonfishes, goatfishes and parrotfishes. The wide seagrass areas on the east side of the island harbor numerous invertebrates, small fish and algae.

Base Yield Figure and Adjustments

Table 2 presents the annual fish yield of Pamilacan Island. The 'market' figure is the total weight counted daily, and is comprised of fish sold through island middlemen, plus some locally consumed fish. Adjustments for those fish not included in the volunteers' fish lists were made as follows:

a) *Consumption.* Ten families followed for one week during each monsoon were found to eat from 4.3 kg/family/week of reef fish in the southwest monsoon (May-October) to 1.6 kg/family/week in the northeast monsoon (November-April). Almost all families ate substantially more reef fish during the southwest monsoon when pelagic fish were scarce. A conservative estimate of 1 kg/family/week was used to compute total reef fish consumed.

b) *Outsiders.* Reef fish captured by outsiders were calculated on a case-by-case basis. During the study year, *muro-ami* fishermen came to Pamilacan once (June 1985) and took an estimated 1,000 kg. Dynamiters came nine times (July 1985; January, February and March 1986) and took an estimated 500 kg altogether.

c) *Gleaning.* Organisms gleaned included sea urchins, sea cucumbers, crabs, eels, algae, fish and molluscs. The amount harvested per gleaner was estimated by measuring the wet weight of material gleaned for 55 randomly selected gleaners. The mean weight, 3.0 kg/gleaner, was halved to eliminate inclusion of rocks, broken corals, and water. Gleaning took place on average 15.8 days/month by an average of 45.2 gleaners.

Locality, Seasons, Income

Reef and nonreef fish. Reef fish are those caught on the reef as measured to the 20-m isobath (see Table 3). Nonreef fish are pelagic fishes not associated with the island and its reef (Table 3).

Seasonal variation. The overall catch was higher in the northeast monsoon, when fishermen pursue rays, sharks and tuna (Fig. 1). During the southwest monsoon when these are not abundant, the majority of the catch comes from the reef. On a yield/area basis, reef catches are highest in the peak of the southwest monsoon, declining through the northeast monsoon (Fig. 2).

Income. During the study year, the value of each daily catch was listed by the fishermen-volunteers at the prevailing prices. The per family mean annual income of ₱1,991 (= US$100) (Table 4) was similar to that of Apo Island. However, unlike at Apo, the Pamilacan fishermen depend on nonreef fish for most (74%) of their earnings.

Discussion

The Pamilacan reef yield (including gleaning) of 17.9 t/km^2/year was computed from actual daily counts of gleaners and fish taken and from conservative estimates of additional consumption. Because catches have been inadvertently overlooked by the fishermen-volunteers and no measure has been made of catches from ten fish traps owned by outsiders, the yield figure presented for Pamilacan should be read as conservative.

If gleaning is included, this compares favorably with those found in the earlier mentioned studies. However, it is considerably lower than the heavily gleaned Samoan reef reported by Wass (1982) unless the Pamilacan reef area were reduced to only that portion gleaned. Perhaps the most significant comparison is with nearby Apo Island, with a reef yield of 22.1 t/km^2/year (Table 2).

The physical nature of Pamilacan's reef clearly influences its yields. The large, gradual sand and seagrass slope offers a rich area for gleaning, which contributes 7.1 t/km^2/year, or 40%, of the total reef yield. Little gleaning, in contrast, is done at Apo Island. Pamilacan's relatively poor coral reef community provides a suboptimal habitat for reef fishes; this is exacerbated by destructive fishing. If gleaning and jacks (which in many studies are considered pelagic fish) are not included, Pamilacan's reef yield is 9.8 t/km^2/year.

Fishing strategy also influences reef yields at Pamilacan. While Apo Island fishermen obtain 80% of their cash income from reef fish, Pamilacan fishermen obtain only 26% of their income from reef fish. They seem to fish the reef primarily for subsistence, and they increase their efforts on the reef only during June-September, when runs of pelagic fish are light. Their gear reflects this predisposition, since capture nets for manta and sting rays are common, while specialized nets for catching fusiliers are rare. Underwater fish counts suggest some reef fishes, such as fusiliers, may be underexploited on Pamilacan (Tables 1 and 3).

The implication of the Pamilacan data is that reef yields reported elsewhere may underestimate true reef yield potentials, especially on those reefs of good quality. The Philippines, with an extremely high fishing intensity by small-scale, local fishermen, is a logical place to discover high yields, even on inferior reefs.

Acknowledgements

The study was conducted under the auspices of the Marine Conservation and Development Program, a joint project of USAID-The Asia Foundation, Manila, and Silliman University, Dumaguete City. The unique, participatory method of data collection would not have been possible without the assistance of Abundia Adanza and Evelyn Torrequemada, MCDP social workers who lived on Pamilacan for the entire study period. Alexandro Ansula and Henrietta Nalum-Zema lived and worked on Apo Island. Computer coding of the voluminous catch records and production of monthly reports fell on the capable shoulders of Lurli Teves and Mariter Dales. Much credit, finally, goes to the many Pamilacan and Apo fishermen who were interested enough in this project to record their catches for the year.

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Table 1. Comparison of fish diversity and abundance for the reefs of Pamilacan and Apo Islands.[+]

	Pamilacan	Apo
Mean no. of species/750 m^2	n = 15	n = 10
*Acanthurids (surgeons)	7.7	8.6
Chaetodontids (butterflies)	6.1	10.0*
Labrids (wrasses)	5.1	6.4
*Caesionids (fuliliers)	1.7	1.9
*Serranids (groupers)	0.5	0.2
Pomacentrids/Anthids	11.7	14.5
*Lutjanids (snappers)	1.2	1.2
All species	34.0	42.8
Mean no. of individuals/750 m^2		
*Acanthurids (surgeons)	228.8	304.7
Chaetodontids (butterflies)	33.7	120.2**
Labrids (wrasses)	50.0	126.6
*Caesionids (fusiliers)	560.4	221.1*
*Serranids (groupers)	0.5	0.2
Pomacentrids (damsels)	(1,075.6)	(750.6)[++]
Anthids	303.7	476.1
*Lutjanids (snappers)	5.3	7.6
*Carangids (jacks)	1.4	0.8
*Nemipterids (breams)	6.4	0.6
*Mullids (goats)	57.0	31.8
*Scarids (parrots)	97.7	117.0
Zanclids (moorish idol)	7.9	11.4
All groups[++]	1,352.8	1,418.1
*Important food fishes	957.5	683.8

[+] Censuses made between 6 and 15-m deep in both steep and gradual slope habitats.

[++] Pomacentrids not included in total because a large standard deviation and absolute value tends to obscure the results.

*$p < .05$. ** $p < .01$.

Table 2. Summary of reef and nonreef fish yields (catch for market and consumption, gleaning and outsider catch) for Pamilacan and Apo Islands, April 1985-March 1986.

	Pamilacan	Apo
Reef catch (kg)		
Market	8,464	11,334
Consumption*	9,360	4,160
Outsider	1,500	n/a
Miscellaneous gleaned	12,872	0
Total	32,196	15,494
Nonreef catch (kg)		
Total	25,996	4,874
Total catch (kg)	58,192	20,368
Reef yield (t/km^2/year		
Reef area (km^2)**	1.8	0.7
Total reef	17.9	22.1
Reef yield without gleaning	10.7	22.1
Carangids in nonreef catch***		
Market reef	6,703	7,551
Total reef	30,435	11,711
Total nonreef	27,757	8,657
Reef yield (t/km^2/year)	16.9	16.7
Reef yield without gleaning	9.8	16.7

*Kg consumed determined to be approximately 1 kg/family/week of reef fish on each island.

**To 20-m isobath.

***An alternative calculation including carangids in the nonreef catch since they are semi-pelagic fishes.

Table 3. Weights and composition of major fish families caught on reef and nonreef areas surrounding Pamilacan and Apo Islands, April 1985-March 1986.

	Pamilacan		Apo	
	kg	%	kg	%
Market reef catch				
Carangids (jacks)	1,761	21	3,783	33
Cephalopods	1,447	17	400	4
Acanthurids (surgeons)	1,126	13	3,866	34
Lutjanids (snappers)	1,096	13	425	4
Scarids (parrotfishes)	1,006	12	453	4
Caesionids (fusiliers)	602	7	1,617	14
Sphraenids (barracudas)	340	4	145	1
Lethrinids (emperor breams)	273	3	17	<1
Serranids (groupers)	188	2	92	<1
Pomacenthrids (damsels)	102	1	192	2
Kyphosids (rudder-fishes)	84	<1	117	1
Nemipterids (breams)	80	<1	51	<1
Plectorhynchids	69	<1	0	
Mullids (goatfishes)	66	<1	29	<1
Siganids (rabbit-fishes)	23	<1	3	<1
Miscellaneous	200	2	145	1
Market nonreef catch (kg)				
Scombrids (tuna)	7,018	27	3,869	79
Stingrays	3,764	14	52	1
Exocoetids (flying-fish)	3,479	13	3	<1
Scombrids (mackerel)	2,856	11	478	10
Belonids (needle-fishes)	2,809	11	316	6
Sharks	2,601	10	2	<1
Mobulids (manta rays)	2,528	10	0	
Swordfishes	279	1	0	
Elopids (Tarpon)	254	1	96	2
Whale shark	210	1	0	
Coryphaenids (Dolphinfish)	184	<1	24	<1
Miscellaneous	15	<1	46	1

Table 4. Summary of cash income derived from fish catch on reef and nonreef areas and precious shell collection at Pamilacan and Apo Islands, April 1985-March 1986.

	Pamilacan	Apo
Fish		
Reef fish catch	84,037*	116,488
% of total	26	80.1
Nonreef fish catch	238,148	27,516
% of total	74	19.1
Total fish income	322,195	144,004
Precious shells**		
No. collected	180	0
Mean value	200	0
Total shell income	36,000	0
Total cash income	358,308	144,004
Mean income/family	1,991	1,800

*All figures in Philippine pesos (P20 = US$1).

**Most common shells include *Conus gloria-maris*, *Conus* spp. and *Strombus* spp.

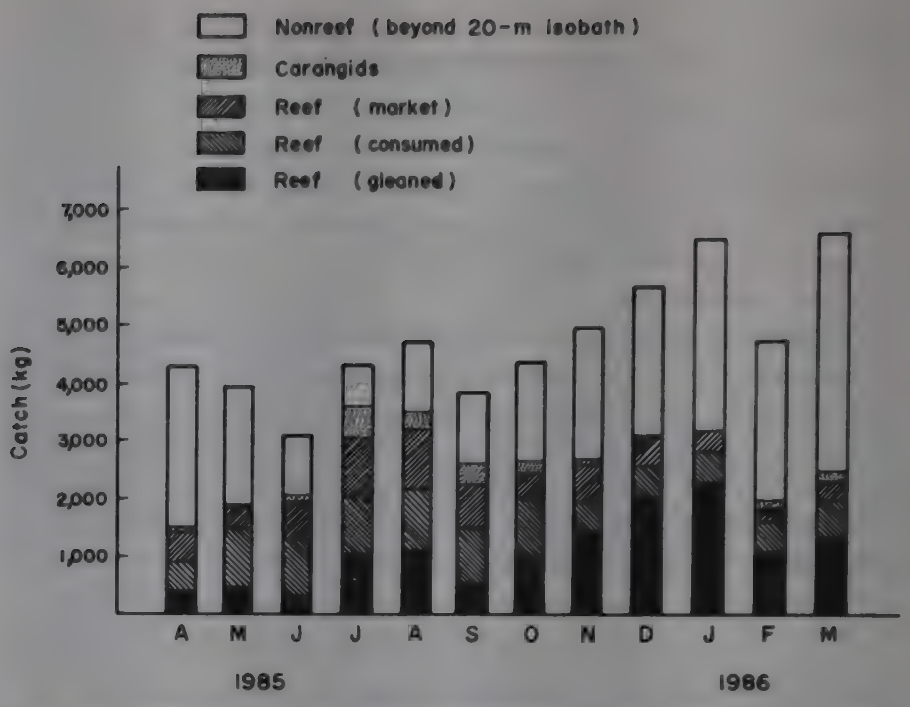

Fig. 1. Total catch per month from the reef and nonreef areas surrounding Pamilacan Island.

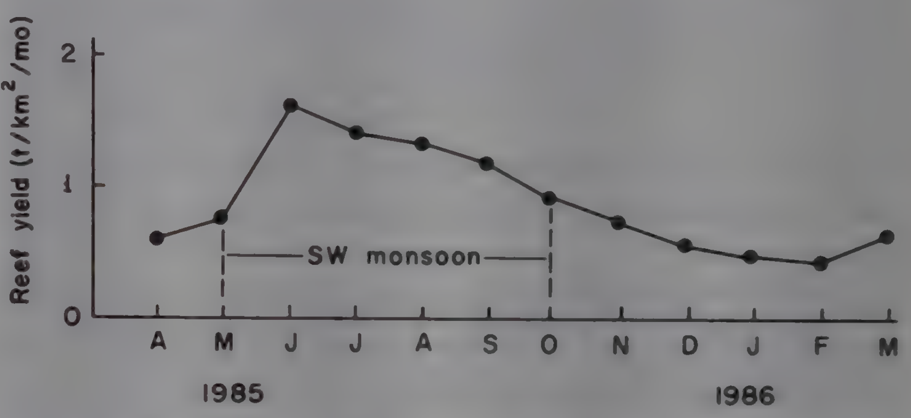

Fig. 2. Reef fish yields per month, Pamilacan Island, April 1985-March 1986.

Yield-Per-Recruit Analysis of Ten Demersal Fish Species from the Samar Sea, Philippines

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SILVESTRE, G.T. 1986. Yield-per-recruit analysis of ten demersal fish species from the Samar Sea, Philippines, p. 501-504. *In* J.L. Maclean, L.B. Dizon and L.V. Hosillos (eds.) The First Asian Fisheries Forum. Asian Fisheries Society, Manila, Philippines.

Abstract

Growth parameters (L_∞ and K) of the von Bertalanffy equation and mortality coefficients (Z, M and F) of the exponential decay model are presented for ten trawl-caught fishes from the Samar Sea, Philippines. These parameter estimates were utilized in yield-per-recruit analyses to evaluate prevailing exploitation rates and the impact of 2-cm cod-end mesh size used by trawlers in the area.

Relatively high growth rates and mortality coefficients typical of most short-lived tropical fish species were obtained. They imply relatively fast turnover rates allowing fishing pressure maintained at relatively high levels. It is shown that the cod-end mesh size of 2 cm is much too small for the mix of ten species studied.

Introduction

The Samar Sea, off the western coast of Samar island in the eastern Visayan region, is an important traditional fishing ground in the Philippines. In 1976, approximately 27,000 t of fish and invertebrates were landed from the area, about 22% of which were caught by commercial trawlers, i.e., by craft of more than 3 t. In the latter part of 1976, commercial trawlers and purse seiners were banned by the national government from operating in areas less than 13-m deep and/or within 7 km from the coast. Statistics indicate that total production from the area subsequently went down by 29% from the 1976 total to an average of 19,200 t in the 1977-1981 period. Catches by the commercial fisheries similarly declined to 16% of the total (Armada et al. 1983). The partial closure was in response to sociopolitical considerations. However, management of the fishery resources in the Philippines suffers from lack of appropriate biological information. For instance, the only studies done on Samar Sea are those by Warfel and Manacop (1950) and Legasto et al. (1975).

To help fill the need for appropriate biological information, the College of Fisheries, University of the Philippines in the Visayas, in coordination with the National Science Technology Authority and the German Agency for Technical Cooperation (GTZ), conducted a trawl survey of Samar Sea from March 1979 to May 1980 (Armada et al. 1983). This paper presents the results of an investigation of the population parameters of the more abundant species in the catch during the survey. With methods of analysis of length distributions improved in the last several years, the growth parameters (L and K) of the von Bertalanffy equation and mortality coefficients (Z, M and F) were estimated for ten trawl-caught fish species. These ten species (Table 1) comprised approximately 43% of the total catch of approximately 52,000 kg of fish and invertebrates during the survey. The parameter estimates were subsequently utilized in yield-per-recruit (Y/R) analysis to evaluate prevailing exploitation rates and to assess the impact of the 2-cm cod-end mesh size commonly used by trawlers in the area.

Methods

Fish growth is conventionally described in fisheries work by the von Bertalanffy growth equation, i.e., $L_t = L_\infty (1-e^{-K(t-t_o)})$, where L_t is the length at age t; L_∞ the asymptotic length; e the base of Napierian logarithm; K the growth coefficient, and to the theoretical age at zero length. In turn, the decrease in number through time of a cohort/population is described as an exponential decay process, namely, $N_t = N_o e^{-Zt} = N_o e^{-(F+M)t}$, where N_t is the number surviving at time t; N_o the initial number; and Z the total instantaneous mortality coefficient of which F and M, respectively, are its fishing and natural mortality components. In this study, L_∞ and K were estimated using ELEFAN I (Pauly et al. 1983a) with to taken as equal to zero for all species. The parameter Z was estimated via ELEFAN II (Pauly et al. 1983b), while M was computed from the empirical equation of Pauly (1980a) relating M, L_∞, K and mean environmental temperature. The estimate of F was obtained by subtraction of M from Z. The exploitation ratio, E (Beverton and Holt 1966), was then computed from the expression E = F/Z.

Y/R was calculated by the expression of Paulik and Gales (1964) which accounts for nonisometric growth and involves polynomial approximation of the incomplete beta integral. Y/R response surfaces for each of the species were generated with the following considerations: (1) F and c variables ($c = l_c/L_\infty$, l_c being the length corresponding to the mean age at first capture, t_c); (2) $t_o = 0$, and; (3) t_m, the maximum relative age, infinitely large (i.e., $e^{-K\,(t_{mm}-t_o)} = 0$). The eumetric fishing line (EFL) B - B' in the graph of Beverton and Holt (1957) was then subsequently identified for each species.

Conversions from c to mesh size (Ms) were made by using selection factors (S.F.), computed for each species, i.e., Ms = L_c/S.F. (Gulland 1969). The S.F. for the first three species in Table 3 were estimated from covered cod-end selection experiments in the Samar Sea (Silvestre 1984). The rest were taken from the average of S.F. values for the species from other areas in the South China Sea (Jones 1976; Sinoda et al. 1979). The W values were obtained from L_∞ using the length-weight relationships given by Villoso and Hermosa (1980).

Results

Estimates of L_∞ and K from ELEFAN I are given in Table 1. Relatively high K and low L_∞ values typical of most short-lived tropical fish were obtained (Pauly 1978). Good fits to the length distributions and restructured frequencies (ESP/ASP ratios of 0.55-0.91, see Pauly and David 1981) were produced by the growth parameter estimates for the species considered.

The number of recruitment pulses (i.e., peaks in recruitment), based on the cohorts that can be followed in the length distribution, was either one or two per year (Table 2). Two peaks per year were observed for *L. bindus, L. splendens, P. tayennus* and *U. moluccencis*. Single annual peaks were observed for the other six species.

The mortality rates computed from ELEFAN II for each of the ten species are also given in Table 2. Good fits to the descending right-hand limb of the catch curves were obtained, with correlation coefficients for the regressions varying between 0.97 and 0.99. Relatively high mortality rates were obtained for the species investigated. Estimates of Z varied between 1.27 *(Nemipterus japonicus)* and 4.28 *(Leiognathus bindus)* while M ranged from 1.05 *(N. japonicus)* to 2.21 *(L. bindus)*. Such high values of M imply relatively fast turnover rates allowing maintenance of F at similarly high levels. The highest estimated values of F were those on *L. bindus, Upeneus moluccencis* and *L. leuciscus*. The lowest were those on the nemipterids *N. japonicus* and *N. nematophorus*.

The W_∞, K and M values utilized in Y/R calculations for the ten species are given in Tables 1 and 2. The EFLs for all species investigated show the lack of a distinct point at which Y/R is maximized. Along the EFL for each species, Y/R theoretically increases with increasing F until E is approximately equal to 1. The lack of a distinct maximum is commonly addressed by taking E = 0.5 as the optimum level of exploitation. This is under the assumption that Y/R is maximized without undue risk to the stock when F is approximately equal to M (Gulland 1971; Pauly 1980b). The E values for each of the ten species are given in Table 2; they varied between 0.15 and 0.53. Based on the E = 0.5 optimization criterion (and categorically setting $0.40 \leq E < 0.55$ as sufficiently close to the optimum, $0.20 \leq E < 0.40$ as moderately underexploited, and $E < 0.20$ as considerably underexploited), the fishery operating in the Samar Sea was: (1) considerably underexploiting *Nemipterus japonicus;* (2) moderately underexploiting *Pentaprion longimanus, N. nematophorus* and *L. splendens*, and (3) exploiting at or close to the optimum the rest of the other six species.

The range (hatched area) of the EFLs in the F,c plane for all the ten species is shown in Fig. 1. The range is bounded by the EFL of *L. splendens* (lower) and *Priacanthus tayennus* (upper). The points in the plane at prevailing Fs in Table 2 and cs corresponding to Ms = 2.0 cm for all the 10 species are plotted as numbered circles in the figure. It is apparent that growth overfishing occurs for all species at 2-cm mesh size given prevailing Fs.

The optimum F (F_{opt}) values corresponding to E = 0.5 for the ten species are given in Table 3, the range of which (0.64-2.14) is marked on the F-axis of Fig. 1. The optimum c (c_{opt}) values corresponding to F_{opt} are also given in Table 3 with the range (0.42-0.46) marked on the c-axis of Fig. 1. The c_{opt} range in the figure emphasizes the inappropriateness of the 2-cm mesh size. Conversion of c_{opt}'s to mesh size (Ms_{opt}) using S.F.'s further emphasizes the point. Ms_{opt} varied between 3.0 and 6.9 cm, implying that a mesh size of 2 cm is unjustifiable for the mix of ten species considered in the study.

Estimates of M from Pauly's (1980a) empirical equation are acknowledged to be approximate. An attempt, hence, to consider variations in M is given below.

Values of M for each of the ten species were incremented and decremented by 25%, respectively. Increasing M depresses the location of the EFLs and vice versa. Figs. 2 and 3 show the consequent range of EFLs and location of the ten species in the F,c plane at 25% decrement and increment, respectively, on M. Table 3 gives the corresponding optimum mesh sizes when M is decremented (Ms'_{opt}) and incremented (Ms''_{opt}) by 25%. Ms'_{opt} varied between 3.3 and 7.8 cm while Ms''_{opt} ranged from 2.7 to 6.3 cm. Despite the imposed variation on M, it is evident that a mesh size of 2 cm is inappropriate.

The permissible variation on M (decrement and increment) to allow conclusions as to prevailing exploitation status made in the previous section are given in Table 4. Permissible M increments varied between 4.8% and 28.5% while decrements varied between -3.6% and -21.4%. Conclusions as to prevailing exploitation on *L. equulus* and *L. leuciscus* are more sensitive to underestimation of M. Conclusions as to prevailing exploitation state are valid to the extent that variability of M remains within such bounds.

Discussion

All available information confirms that the mesh size of 2 cm presently used by Samar Sea trawlers is too small. The optimum range of mesh sizes so far identified is 3-6 cm (see also Silvestre 1984, 1986). The newly proposed model of Pauly and Soriano (this vol.) and the fact that most Samar Sea fish caught by trawlers are rather small suggest that the optimum mesh size for the multispecies stock discussed here, and probably for other stocks in the Philippines, is in fact nearer to the upper than to the lower limit of this range.

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Table 1. Relative abundance and growth parameters of ten trawl-caught species from the Samar Sea, Philippines.

	Species name	Relative abundance (%)	L_∞ (FL, cm)	K (annual)	W_∞ (g)
1	*Leiognathus bindus*	24.1	12.1	0.98	44
2	*Pentaprion longimanus*	5.6	14.1	0.70	72
3	*Nemipterus nematophorus*	3.0	25.5	0.43	294
4	*Leiognathus splendens*	2.8	13.1	0.90	63
5	*Leiognathus equulus*	2.2	24.0	0.56	380
6	*Priacanthus tayennus*	1.8	29.0	0.65	293
7	*Selaroides leptolepis*	1.0	19.9	0.53	158
8	*Nemipterus japonicus*	0.9	26.6	0.45	340
9	*Upeneus moluccencis*	0.8	24.1	0.75	276
10	*Leiognathus leuciscus*	0.5	13.7	0.93	39

Table 2. Number of recruitment pulses, mortality parameters and exploitation ratio for ten trawl-caught species from Samar Sea, Philippines. Species names as in Table 1.

Species no	NRP	Z (annual)	M (annual)	F (annual)	E (annual)
1	2	4.28	2.21	2.07	0.48
2	1	2.53	1.69	0.84	0.33
3	1	1.50	1.05	0.45	0.30
4	2	3.13	2.02	1.11	0.35
5	1	2.20	1.26	0.94	0.43
6	2	2.47	1.34	1.13	0.46
7	1	2.64	1.29	1.35	0.51
8	1	1.27	1.08	0.19	0.15
9	2	3.47	1.62	1.85	0.53
10	1	3.86	2.12	1.74	0.45

Table 3. Selection factors and optimum F, c and Ms for ten trawl-caught species from Samar Sea, Philippines. Species names as in Table 1.

Species no.	F_{opt}	c_{opt}	S.F.	Ms_{opt} (cm)	Ms'_{opt} (cm)	Ms''_{opt} (cm)
1	2.21	0.46	1.56	3.6	4.0	3.3
2	1.69	0.46	2.00	3.2	3.4	2.7
3	1.05	0.42	2.25	4.8	5.2	4.1
4	2.02	0.42	1.68	3.3	3.7	3.0
5	1.26	0.42	1.59	6.3	7.2	5.7
6	1.34	0.46	1.94	6.9	7.8	6.3
7	1.29	0.42	2.45	3.4	3.9	3.1
8	1.08	0.42	2.24	5.0	5.5	4.3
9	1.62	0.44	2.37	4.5	5.3	3.9
10	2.12	0.44	2.05	3.0	3.3	2.7

Table 4. Limits to permissible variation on M, for 10 species (see Table 1) in the Samar Sea, Philippines.

Species no.	Lower limit (%)	M	Upper limit (%)
1	−12.9	2.21	22.5
2	−10.0	1.69	19.8
3	−14.1	1.05	14.3
4	−6.9	2.02	24.0
5	−21.4	1.26	4.8
6	−17.1	1.34	10.6
7	−7.9	1.29	22.8
8	−5.8	1.08	17.6
9	−3.6	1.62	28.5
10	−18.1	2.12	9.2

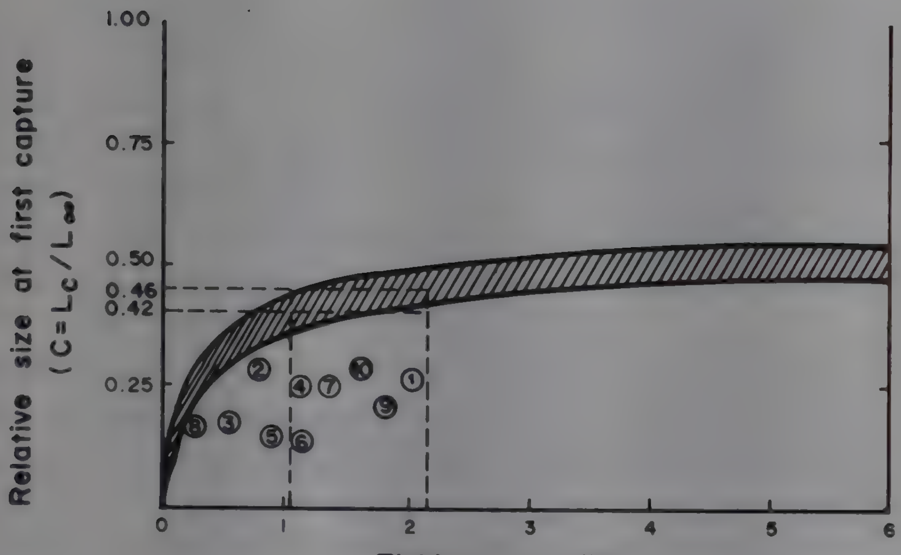

Fig. 1. Range of eumetric fishing lines (hatched area) and location of the 10 species in the F, c plane. Species as in Table 1.

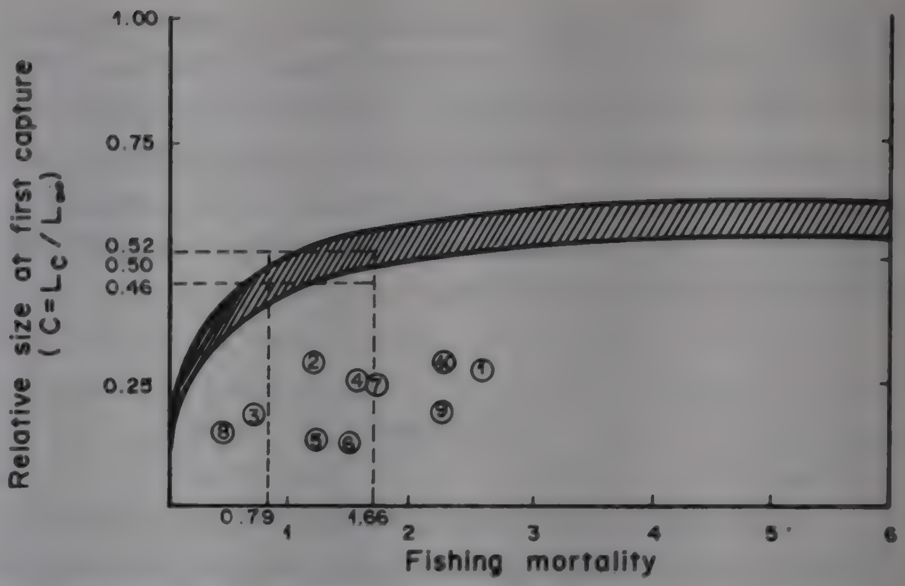

Fig. 2. EFL range (hatched area) and location of the 10 species (see Table 1) in the c, F plane with M decremented by 25%.

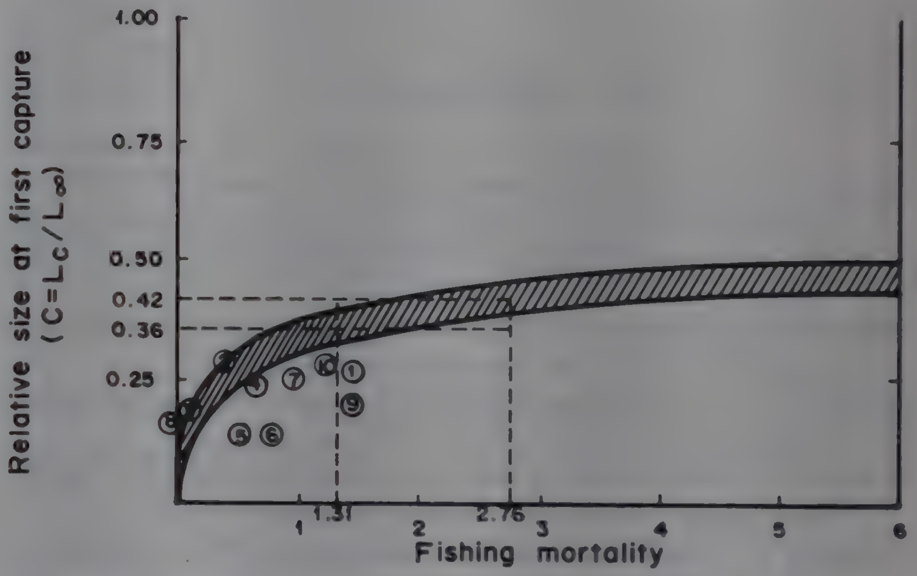

Fig. 3. EFL range (hatched area) and location of the 10 species (see Table 1) in the c, F plane with M incremented by 25%.

Effects of Environmental Variability and Fishing Pressure on the Catches of Penaeid Shrimp in the Gulf of Carpentaria, Australia

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Abstract

Catches of several major penaeid shrimp fisheries in the Gulf of Carpentaria, northern Australia, have shown steady decline over the past decade. One aspect of the research on recruitment of these shrimp has been aimed at separating the effects of natural from man-induced changes in these populations. In the case of the banana shrimp, *Penaeus merguiensis*, in the southeastern Gulf, the decline appears to have been mainly environmentally induced. High rainfall stimulates a large proportion of the juvenile shrimp resident in the mangrove estuaries to emigrate offshore into the commercial fishery and good catches follow. The underlying stock-recruitment changes have also been examined using simple stock-recruitment models. Less is known about the tiger shrimp, *P. esculentus* and *P. semisulcatus*, but these species are presently under investigation. The juvenile nursery areas of both species are seagrass/algal beds and long-term sampling of juvenile shrimp and seagrasses have been initiated. Population dynamics of offshore stocks are also being studied and should assist in determining the role of environmental changes, if any, in these declining stocks.

Introduction

The major fisheries for penaeid shrimp occur in the tropical regions of Australia, centered around the Gulf of Carpentaria. Three species groups make up the bulk of the tropical catch. These are the banana shrimp, *Penaeus merguiensis*, the tiger shrimp, *P. esculentus* and *P. semisulcatus*, and the endeavor shrimp, *Metapenaeus endeavouri* and *M. ensis*. Two distinct fishing modes occur for these species groups. In the first, schools of banana shrimp are located by searching with echo sounders or spotter aircraft and then fished intensively by all boats in the region, usually during daylight. In the second fishery, more traditional trawling is carried out, usually at night, on recognized productive tiger/endeavor (mixed species) shrimp grounds. The two fisheries are partially separated in both space and time allowing fishing vessels to operate in both sequentially. The banana shrimp fishery season lasts only a few weeks after a predetermined opening date. The mixed species fishery operates for much of the year with a seasonal peak in catch occurring approximately six months later than that of the banana shrimp fishery. Both fisheries operate under a limited license management regime and employ seasonal closures to optimize yield.

The shrimp fisheries commenced in the late 1960s, and for the first decade, banana shrimp catches dominated the total catch. With increasing effort and discovery of new fishing grounds, tiger shrimp catches increased in relative importance and now form the main component. Fishing effort in the banana shrimp fishery has remained fairly constant since the early 1970s but catches have been characterized by wide interannual fluctuations with a downward trend. The total catch of banana shrimp is a good index of recruitment because of the very high exploitation rate and short season of fishing. Changes in annual effort affects the length of time that it takes the fleet to catch the available shrimp but does not affect the total catch. Both effort and catches in the mixed species fishery increased steadily during the 1980s (Fig. 1), but the catch per unit effort has been declining steadily since the mid-1970s. Neither total catch nor recorded landings adequately reflect recruitment dynamics of the component species. In a preliminary yield-per-recruit analysis, recruitment was shown to be declining only during the last three to five years, the decline varying in different areas of the Gulf and also varying for the different species making up the fishery (Somers, pers. comm.).

In Australia, prior to 1975, little was known concerning even the basic life history dynamics of the major species. A major program by the Commonwealth Scientific and Industrial Research Organisation (CSIRO) was set up to explain factors which affect fluctuations and trends in production and commercial yield (Dall 1985). The research has been team oriented, covering ecological studies on all life history stages, stock assessment, behavior and physiology along with aspects of the biotic and abiotic environment. In this paper, the present status of our knowledge with respect to interannual variability and longer-term trends in the fisheries and their relevance to management policies are presented.

Banana Prawn Fishery

Life history studies. Penaeus merguiensis spawns offshore, followed by a juvenile nursery period in mangrove estuaries and an offshore spawning migration to complete the cycle. Each of the major life history stages has been studied by different members of our team. A synthesis of these results are given by Rothlisberg et al. (1985). The life history of *P. merguiensis* is rather complex and does not appear to follow the typical *Penaeus* pattern hypothesized by Garcia (1985). Spawning occurs throughout the year but with two periods of increased activity in spring (March-April) and autumn (September-November) (Crocos and Kerr 1983). However, because of seasonal differences in the transport of larvae as well as differences in the settlement success of postlarvae, it is the spring generation which contributes most offspring into the commercial fishery in the southern Gulf (Rothlisberg et al. 1983; Staples and Vance 1985). The generation time of *P. merguiensis* is six months but because of seasonal changes in larval and postlarval survival, coupled with the effect of only one wet season each year, commercial fishing is confined to autumn. The life history in the northern Gulf is not as well known. The species also spawns twice a year in this region, but in contrast to the southern Gulf, postlarvae from both the spring and the autumn generations recruit into the estuaries and contribute to the fishery. A much wider size and age range of shrimp exists offshore, therefore, and the dynamics of the population are much more complicated than in the south.

Environmental correlative studies. In a first attempt to determine the likely causes of the large interannual variation in catches, the relationships between catches and several environmental variables in six major regions of the Gulf were examined by Vance et al. (1985). Rainfall was found to be positively correlated with catch in the southern (Fig. 2) but not in the northern Gulf. Using stepwise multiple regression techniques it was found that temperature and wind parameters became more highly correlated with catches in more northern regions.

Population ecological studies. Detailed ecological studies on recruitment processes of juvenile and subadult shrimp were first studied in the Norman River estuary located in the southeastern Gulf. A four-year study by Staples and Vance (1985, 1986) examined the dynamics of postlarval immigration and emigration of juveniles to the offshore region over a range of time scales. Interannual variation in the spring recruitment of postlarval *P. merguiensis* was found to be associated with changes in the amount of rainfall recorded in the previous year's rainfall (possibly effected through nutrient influence on larval survival and growth) and the number of adult shrimp present offshore in spring. Despite these changes in postlarval recruitment and subsequent juvenile population numbers, however, the number of emigrants from the estuary each year was largely independent of juvenile densities but closely associated with changes in rainfall (Fig. 3). Rainfall was found to stimulate emigration, the effect being size dependent.

Population modelling. To extend the observations during the four years of ecological studies in the southeastern Gulf, a further five years of juvenile abundance estimates plus 10 years catch statistics were incorporated into the development of a multistage model by Staples (1985). The model provided an excellent conceptual framework to describe why rainfall is the driving force in the system and why factors affecting the abundance of earlier life history stages have little effect on the commercial catch (Fig. 4). Provided the escapement of adult shrimp from the autumn fishery is sufficient to produce enough juveniles somewhere along the plateau of the flat-topped curves in Fig. 4c, the rainfall during the juvenile life history stage will determine the subsequent adult catch. Spawning stock and juvenile numbers only become important if they drop to levels where they fall on the ascending arms of the curves. By comparing the observed and expected catches estimated on the basis of each year's rainfall in the southern Gulf, the downward trend in catches observed since 1974 can be adequately explained by the trend in rainfall over the same period.

Stock assessment and biological management. Knowledge of the basic parameters of growth, mortality and exploitation (Lucas et al. 1979) has enabled the estimation of the optimal date for opening the fishery to provide the maximum yield and value per recruit (Somers 1985). The optimum opening date has been determined for several years and areas in the Gulf and preseason surveys have been carried out to examine the size composition of shrimp in two successive years in order to assess the feasibility of fine tuning the opening date to allow for year-to-year differences in the timing of recruitment. Although there is no strong biological evidence for recruitment overfishing at present, the fishery is overcapitalized, and a buy-back scheme has been introduced to reduce boat numbers and effective fishing effort. In the banana shrimp fishery, however, total catch will probably not be affected.

Mixed Species Fishery

Life history studies. Life history studies on the four main species making up this fishery *(P. esculentus, P. semisulcatus, M. endeavouri* and *M. ensis)* have not been as extensively studied as *P. merguiensis*. A study on the habitat requirements of the juveniles of these species has demonstrated that all, with the exception of *M. ensis*, have very restricted habitat requirements, the majority of juveniles being found on seagrass/algal beds (Staples et al. 1985). The broad scale distribution of the different species in the Gulf was also found to be closely linked to the distribution of these preferred juvenile habitats. Like *P. merguiensis*, regional differences appear to exist in the life history dynamics of these species. In the northeastern Gulf, postlarvae of both *P. esculentus* and *P. semisulcatus* recruited into seagrass beds in two major pulses, one in spring (September-November) and the other in autumn (March-April). *Metapenaeus ensis* and *M. endeavouri* occupied the estuary during the summer wet season months. In contrast, in the Groote Eylandt area in the northwestern Gulf, both species of tiger shrimp showed only one main period of spring-summer postlarval recruitment. Offshore, spawning and recruitment to the fishery of *P. esculentus* is more prolonged compared with the very seasonal dynamics of *P. semisulcatus* (Buckworth 1985). A more detailed study on the seasonal dynamics of population fecundity will soon be published (Crocos, in press).

Population ecology studies. The population dynamics of *P. esculentus* and *P. semisulcatus* have recently been examined in detail. Data on postlarval recruitment, growth and survival of juveniles, and offshore emigration have been collected on several seagrass sites around Groote Eylandt. Four major seagrass communities were chosen for future study from the types recognized by Poiner et al. (in press) for the whole Gulf of Carpentaria. These are: (1) reef flat communities characterized by *Thalassia;* (2) beach profiles characterized by *Cymodocea* and *Syringodium;* (3) sheltered embayments characterized by *Enhalus;* and (4) river mouth areas dominated by *Halophila* and *Halodule*. Preliminary analyses have shown that the abundance of juvenile prawns is lowest on river mouth communities and highest in the sheltered embayments (Fig. 5). The differences in relative abundances appear to result from differences in both the settlement of postlarvae to these areas and subsequent survival of juveniles. These in turn are related to both the height and amount of seagrass as well as substrate type.

Offshore, it has been possible to determine the substrate preferences for the two tiger shrimp species (Somers, in press) as well as estimates of growth parameters (Kirkwood and Somers 1984). Hill and Wassenberg (1985) have shown that streamer tags did not affect growth but affect mortality and catchability. Temperature and moulting effects on catchability must also be considered in analyzing trawl data (Hill 1985). With this information, it has been possible to examine the long-term trends in recruitment into the adult stocks of both species in different areas of the Gulf using commercial catch statistics which do not distinguish between the two species. More detailed studies of the stock-recruitment relationships for both species have recently been carried out by Somers (pers. comm.). Penn and Caputi (1985) have demonstrated a recruitment overfishing of *P. esculentus* in Exmouth Gulf, which has greatly reduced yields in that fishery. New management regulations are presently being introduced to reduce fishing effort in the Gulf of Carpentaria fishery.

Conclusions

In all these species, the most important biological problem lies in determining the nature of the stock-recruitment relationships, preferably between each of the major life history stages. This is not possible without knowing the seasonal dynamics of each stage so that the correct stock index can be related to the correct recruitment index. Although traditionally, recruitment has been assumed to be independent of stock sizes in many penaeid populations, this would appear to be an extremely rash assumption to base the management of one of the world's major fishery resources. Models incorporating both density and environmental effects will play an important role in the understanding of penaeid shrimp dynamics. These, unfortunately, require a long-term commitment of research funds and personnel. In the Gulf of Carpentaria, for example, despite the relatively large investment of time and effort, major gaps in our understanding still exist. The time has come for a more cooperative line of research involving comparative studies of recruitment processes across the geographic range of the species. If several research teams in different countries, all using standardized methods, could be established, the accumulation of our knowledge of penaeid shrimp dynamics could be greatly accelerated. A truly international collaborative effort is required. Because most of the species are found in many of the countries throughout the Indo-West Pacific, it is hoped that this forum can facilitate the setting up of these studies.

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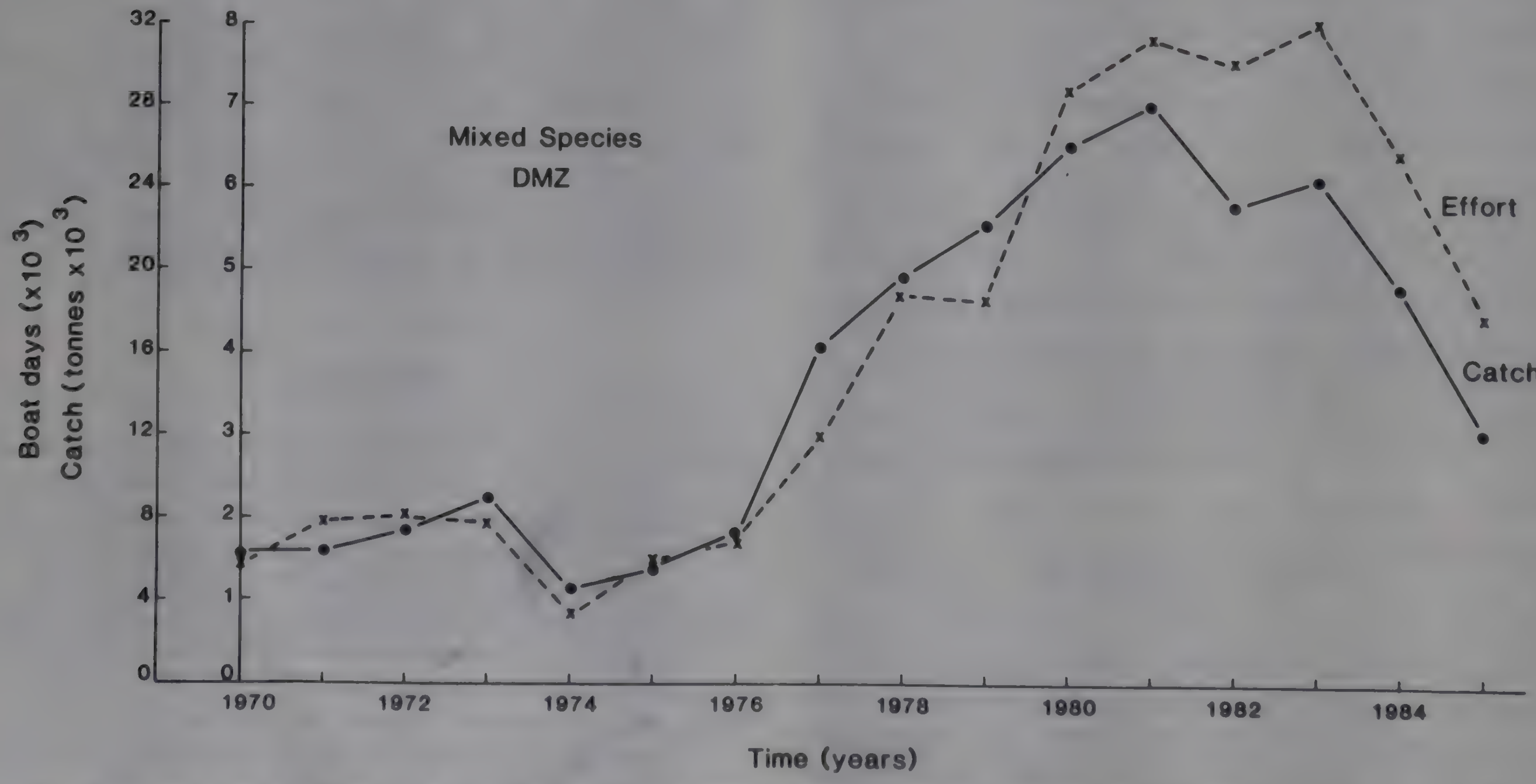

Fig. 1. Annual catch and nominal effort for the mixed penaeid shrimp species in northern Australia (Declared Management Zone). Data of Department of Primary Industry, Canberra, Australia.

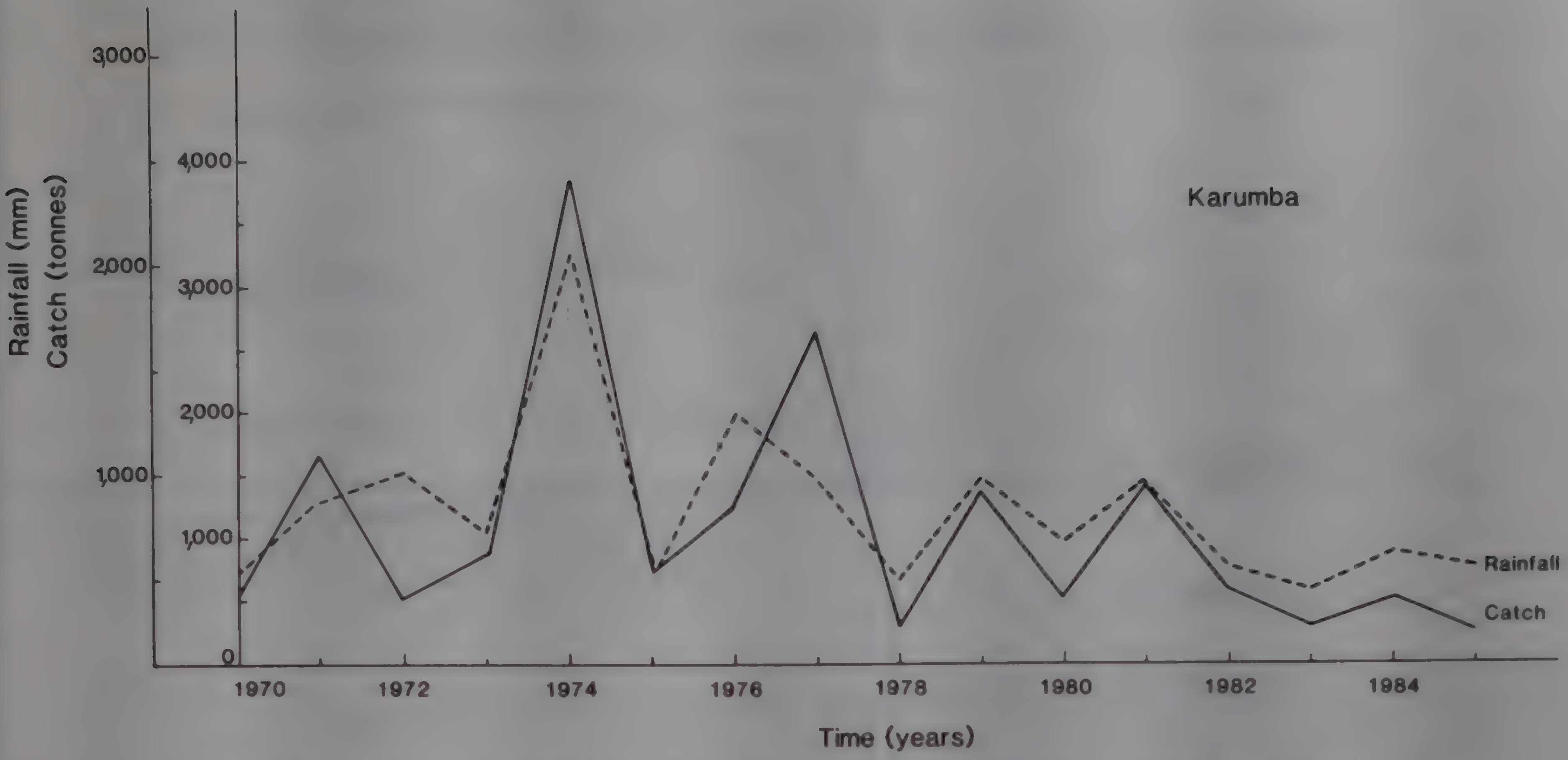

Fig. 2. Annual catch of *Penaeus merguiensis* and rainfall for the Southeastern Gulf of Carpentaria, Australia, 1970 to 1985.

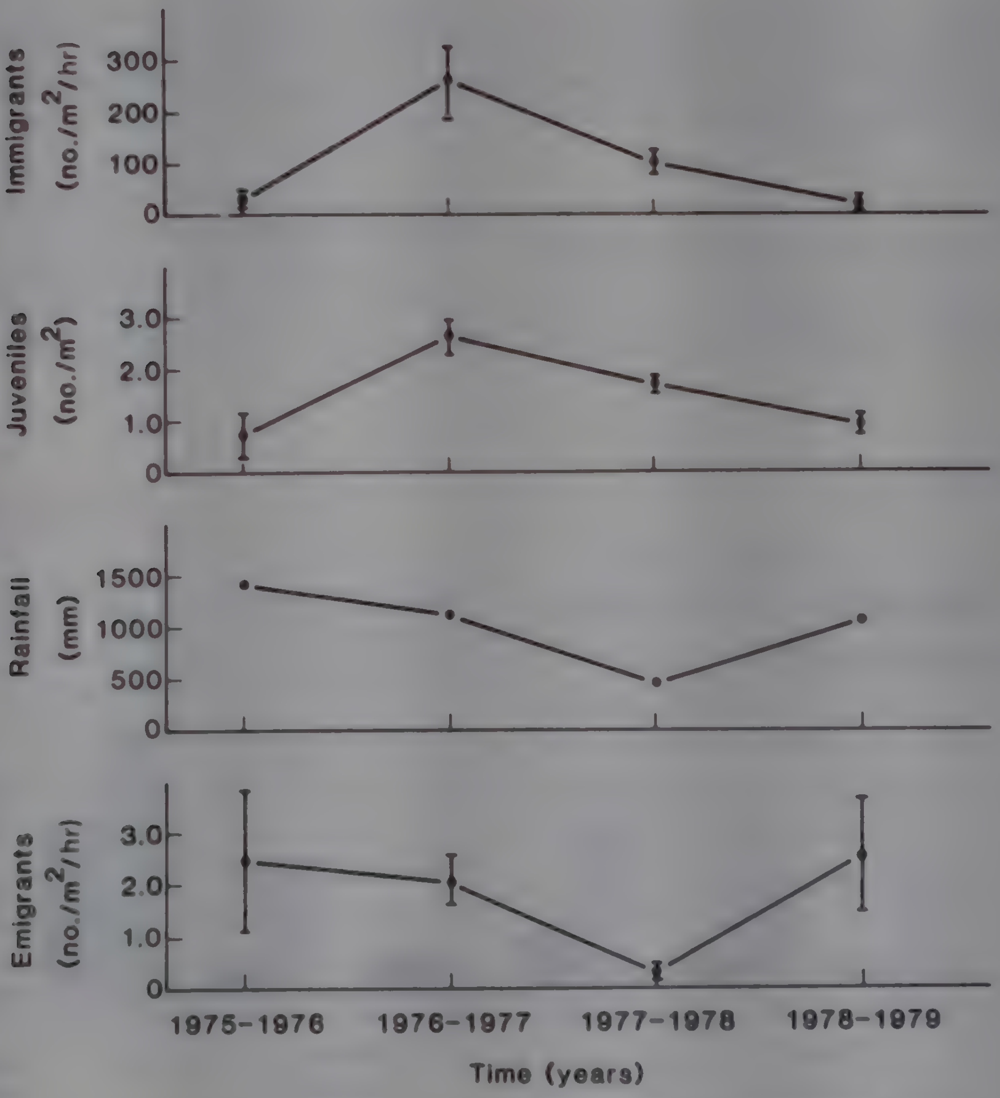

Fig. 3. Interannual variation in the immigration of postlarval *Penaeus merguiensis*, relative abundance of juvenile shrimp, rainfall and emigration of juvenile shrimp from 1975 to 1979. Data from Staples and Vance (1985, 1986).

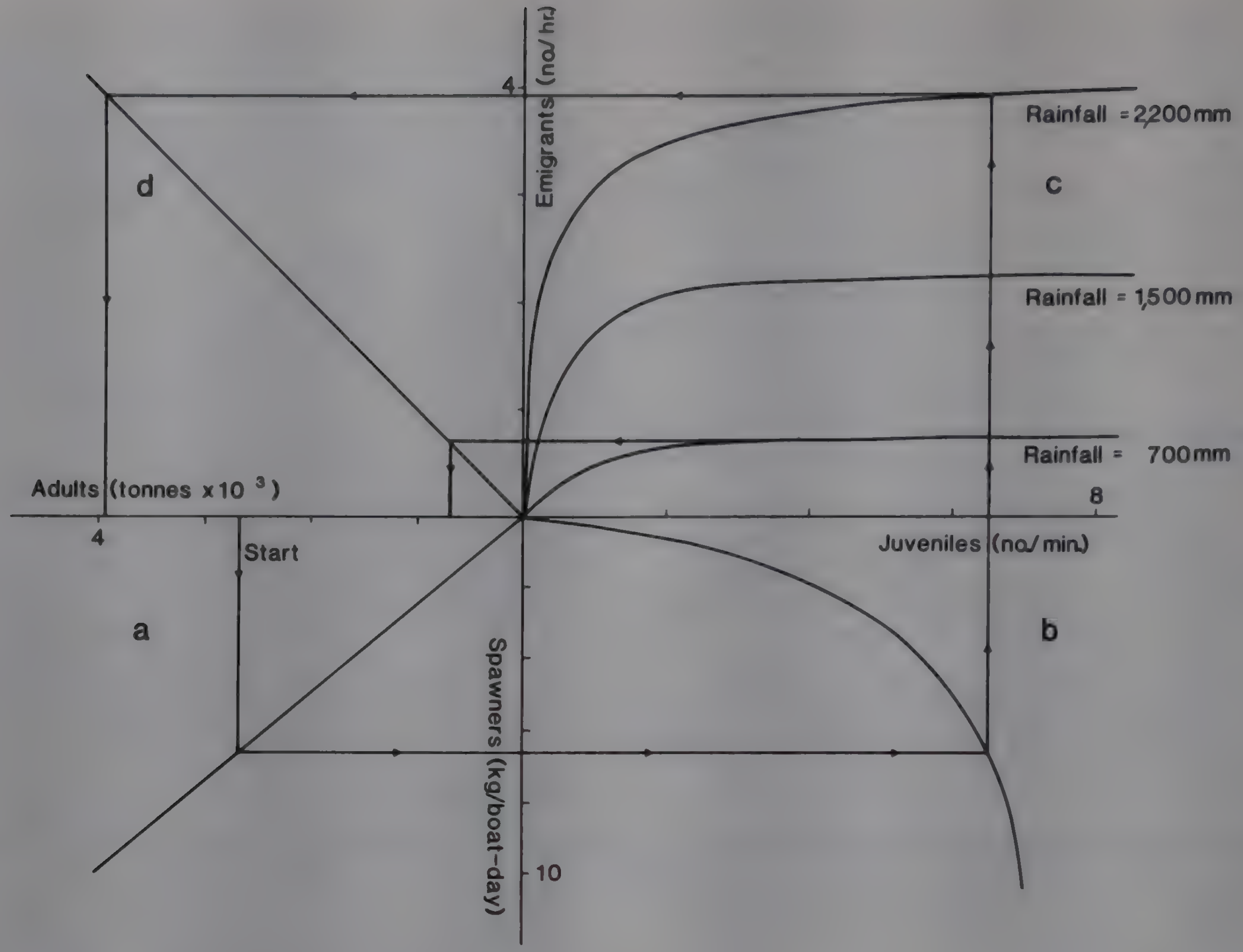

Fig. 4. Multistage recruitment model for *Penaeus merguiensis* from the southeastern Gulf of Carpentaria (a) adult:spawning stock, (b) spawning stock:juveniles, (c) juveniles:emigrants and (d) emigrants:adult catch. Solid lines with arrows trace the change in relative population abundance throughout a life cycle. From Staples (1985).

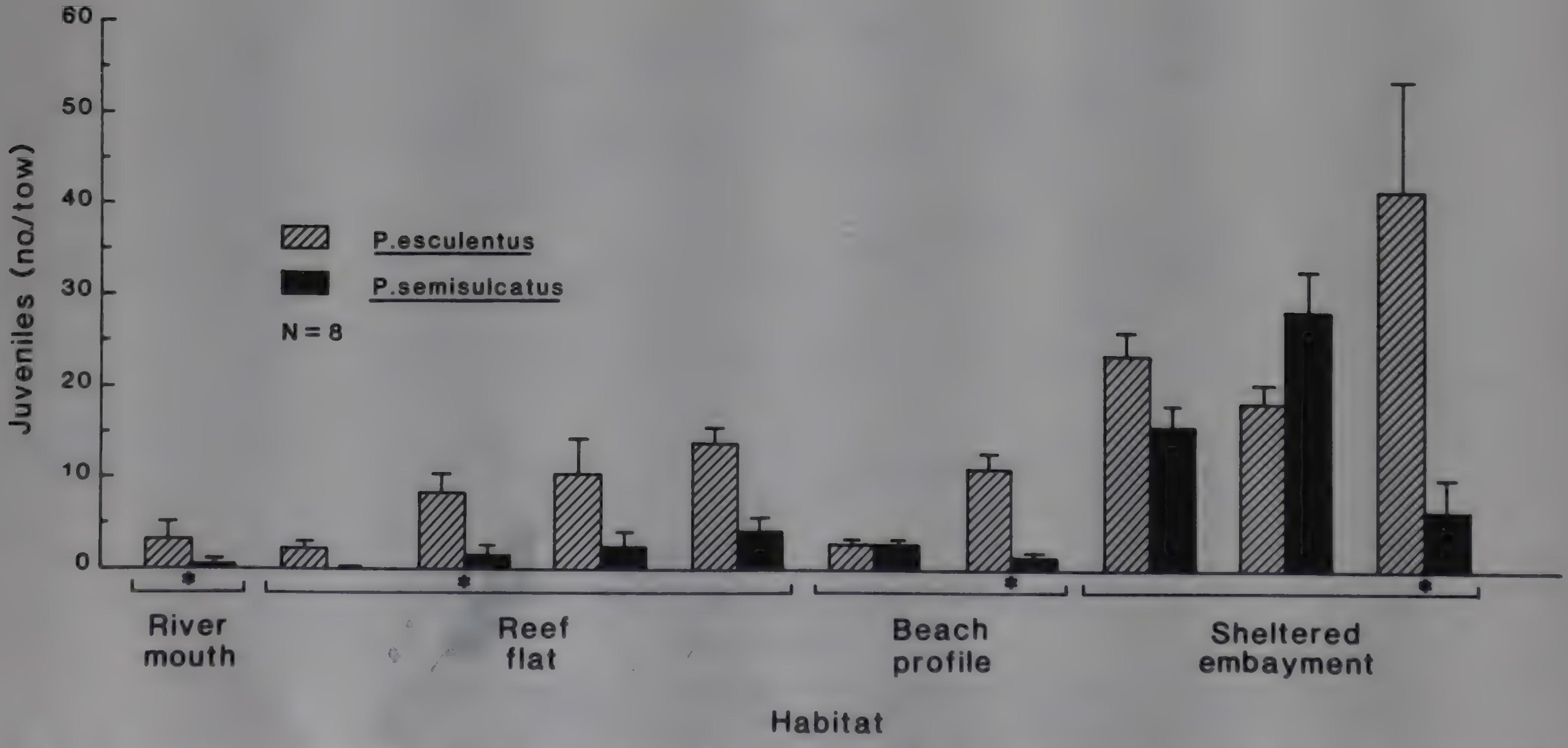

Fig. 5. Relative abundance of the two tiger shrimp species *Penaeus esculentus* and *P. semisulcatus,* taken over one year on differing seagrass habitats around Groote Eylandt in the northwestern Gulf of Carpentaria, Australia.

Biology and Fishery Oceanography of Mackerels and Scads in the Adjacent Waters of Taiwan

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TZENG, W.N. 1986. Biology and fishery oceanography of mackerels and scads in the adjacent waters of Taiwan, p. 511-514. *In* J.L. Maclean, L.B. Dizon and L.V. Hosillos (eds.) The First Asian Fisheries Forum. Asian Fisheries Society, Manila, Philippines.

Abstract

Based upon purse seiner catch data and water temperature, fishery oceanography and biological characteristics of mackerels and scads were analyzed for both northeastern (NE) and southwestern (SW) offshore areas of Taiwan during 1979 to 1985. Mackerels, *Scomber australasicus* and *S. japonicus*, were the most dominant species, which contributed approximately 70% of the total catch. The scads, *Decapterus akaadsi* and *D. macrosoma*, contributed 20-30%. The size composition of *S. australasicus* and *D. akaadsi* was significantly different between the NE area and the SW area. In the NE area the mackerel and scad fishing grounds were in the coastal waters along the boundary to the Kuroshio current during their overwintering migration. The fishing grounds in the SW area were also located in the coastal waters along the boundary to the Kuroshio current during their spring spawning season. The catches of *Scomber* spp. in the NE area were significantly inversely correlated with water temperature.

Introduction

Mackerels *(Scomber japonicus, S. australasicus)* and scads *(Trachurus japonicus, Decapterus* spp.*)* are the most important pelagic commercial fishes in the coastal waters of Taiwan. Their catches have varied from year to year between 20,000 and 40,000 t. Before 1977 most of the mackerels and scads were caught by handliners. Since 1977 most of the handliners have been replaced by large purse seiners. The fishing grounds of mackerels and scads have also extended from the nearshore waters of Taiwan to offshore waters near to Pengchiahsu and Fishing Islands off northeastern Taiwan and near Pratas Islands off southwestern Taiwan.

A large purse seiner consists of five boats: a netting boat, two light boats and two transport boats (Tzeng 1984). Although the large purse seiner is an efficient fishing gear, fishermen spent most of their time searching for fishing grounds. If predictable, the fishermen can save much time searching for fish schools. The formation of the fishing ground of mackerels and scads was known to be related to oceanographic conditions in the Yellow Sea and East China Sea (Tsujita and Kondo 1957; Mori 1978). This paper describes the fishery oceanography, biological characteristics and the fluctuation of the catch of mackerels and scads in the waters adjacent to Taiwan.

Materials and Methods

Beginning 1979, the daily catches, location of fishing ground, fish species, water depth, surface temperature, current direction and velocity and weather were recorded by the captains or crew of the large purse seiners. Based on these data, species composition of the catch and catch per haul (CPUE) were computed for the northeastern area (NE) and southeastern area (SW) off Taiwan.

Fork lengths of 100 to 500 individuals of each species were measured monthly at the Su-Ao fish market from July 1981 to October 1983.

Monthly CPUE and water temperature anomalies were computed. The water temperature was measured at Pengchiahsu by the Taiwan Fisheries Research Institute and at Fishing Island by Nakasaki's Marine Meteorological Observatory of Japan.

Results

The annual catches of mackerels from different fisheries during 1963 to 1984 are shown in Fig. 1. The catch increased from 1963 and reached a peak in 1970. From 1970 to 1974, the catch declined rapidly to a very low level. In 1977, an efficient large type of purse seiner was introduced to Taiwan. The catch of mackerels rapidly increased and reached the 1970 level in 1984.

The annual catches of scads from different fisheries are shown in Fig. 2. 1963 to 1977, most were caught by otter trawl, bull trawl and small purse seiner. After the introduction of the large purse seiner in 1977, the total catch of scads rapidly increased.

The currents, water masses and oceanic boundaries in the East and South China Seas and the fishing grounds of mackerels and scads in the NE area and the SW area are shown in Fig. 3. The fishing grounds of mackerel and scad in the NE area were distributed in coastal waters along the boundary with the Kuroshio current. The Kuroshio penetrated into the lower layer of the coastal waters in the continental shelf of China. A similar situation was found for the SW area. The fishing ground of mackerels and scads in this area was also located in the coastal waters along the boundary to the warm Kuroshio current.

Mackerels included *Scomber japonicus* and *S. australasicus*. These two species are fairly similar in morphology and difficult to distinguish in the field. Therefore, they were combined into a species group as *Scomber* spp. In this study, *Scomber* spp. usually made up about 70% of the catch. The scads included *Trachurus japonicus* and five species of *Decapterus* and made up 20-30% of the catch. A small number of *Etrumeus teres* was taken in the NE area and *Mene maculata* in the SW area in April-June 1983 and 1985 (Table 1).

Monthly length-frequency distributions of the four dominant species, *Scomber australasicus, S. japonicus, Decapterus akaadsi* and *D. macrosoma*, are shown in Figs. 4-7.

The monthly CPUEs were computed for *Scomber* spp., *D. akaadsi* and *D. macrosoma*. The peak catch of *Scomber* spp. in the NE area occurred during December-January. The peak catch of *D. akaadsi* occurred in November. The peak catch of *D. macrosoma* occurred during the September-October period, about two months earlier than that of *Scomber* spp. The fishing season of purse seiners in the SW area was short, from February to May.

The relationship between the catch and water temperature was studied for *Scomber* spp. The peak catch appeared to be from autumn to winter (September-January) when water temperatures were low. The peak catch seemed to be related to the temperature gradient between Fishing Island and Pengchiahsu. The greater the gradient the stronger the oceanic front. As shown previously, the fish schools aggregated near the oceanic front (Fig. 3). CPUEs of *Scomber* spp. were significantly negatively correlated with water temperature ($r = -0.3823$, $P < 0.05$), suggesting that more *Scomber* spp. immigrated into the NE area when the water temperature decreased (Fig. 8).

In addition, the monthly CPUE anomalies (ΔCPUE) and monthly water temperature anomalies (Δ°C) in the NE area were computed and compared. The trend of the frequency distribution of CPUE was opposite to that of Δ °C in the corresponding year. Δ CPUE was lower when water temperature was higher at the coast of Pengchiahsu and Fishing Island in 1983 and 1984 and vice versa in 1982 and 1985.

Discussion

The peak fishing season of mackerels and scads in the NE area was from September to January, corresponding to the period when water temperature decreased. In general, mackerels and scads migrate southward in the autumn and winter and northward in the spring and summer in the East China Sea. Their fishing season in the SW area from February to May was their spawning season (Ku and Tzeng 1985).

The length-frequency distribution of mackerels and scads was significantly different between the NE area and SE area, especially for *S. australasicus* and *D. akaadsi* (Figs. 4 and 6). *S. australasicus* in these two fishing areas might belong to different stocks (Chang and Chen 1976; Ku and Tzeng 1985). However, *D. akaadsi* may belong to the same stock (Chang et al. 1976).

The fishing grounds of *Scomber* spp. were distributed in the coastal waters along the boundary to the Kuroshio current (Fig.3). According to Chu (1970), the China coastal current comes from north China. It flows toward south along the coast when northeast winds prevail during the winter, and retracts back when the southwest monsoon prevails during the summer. A boundary between the Kuroshio current and coastal current develops in the offshore waters of northeastern Taiwan, particularly in the winter. The boundary is characterized by the difference in water properties between the Kuroshio current and coastal current. Its position shifts with season and changes according to the intensity of flow on both currents. The Kuroshio water has high temperature and salinity, while the coastal water has low temperature and salinity. The water temperature anomalies near the coast of Pengchiahsu and Fishing Island were higher in 1983 and 1984 possibly causing the low catch of mackerels and scads in the NE area at that time.

Mackerels and scads always aggregated at the oceanic front (Fig. 3). If fishermen know the location of the oceanic front, they may easily locate the fish schools. Now, the oceanic conditions can be determined quickly by remote sensing techniques and the fishing ground can be predicted.

Acknowledgements

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Table 1. Species composition of the catches of large purse seiners in the offshore areas of northeastern (NE) and southwestern (SW) Taiwan, during July 1982 to September 1985

Species	Fishing ground	Species composition of the catch (%)																
		1982			1983					1984					1985			
		Jul-Sept	Oct-Dec	Total	Jan-Mar	Apr-Jun	Jul-Sept	Oct-Dec	Total	Jan-Mar	Apr-Jun	Jul-Sept	Oct-Dec	Total	Jan-Mar	Apr-Jun	Jul-Sept	Total
Scomber spp.	NE	65.26	72.87	69.98	86.22	39.51	64.34	61.68	62.46	84.80	84.83	67.77	78.24	77.11	94.32	48.28	67.00	72.10
	SW					65.50			65.59	72.30	64.53			60.14	60.56	28.19		36.78
Decapterus macrosoma	NE	16.50	15.87	15.99	0.82	7.90	24.32	5.04	10.81			25.30	19.18	16.59	2.16	22.59	18.49	11.91
	SW					3.92			3.92		17.05			11.64	0.25	5.53		4.11
D. akaadsi	NE	11.22	8.08	7.39	11.62	2.84	3.30	4.61	6.81	12.41	11.22	8.05	1.80	6.61	2.09	12.16	0.91	2.32
	SW					12.34			12.34	23.18	16.13			18.36	7.49	0.76		2.56
D. maruadsi	NE	5.43	2.99	3.67	0.64	13.09	9.64	18.16	10.86	0.82	3.94	1.87	0.62	1.38	0.02	0.34	10.80	5.39
	SW					7.49			7.49		9.42			6.43	4.12	6.29		5.70
D. macarellus	NE	1.50	2.17	1.93	1.80		1.60	7.78	3.98	1.98			0.16	0.37	1.09			0.46
	SW					1.93			1.93	4.53	0.26			1.61	11.49	1.09		3.86
D. tabl	NE	0.09	0.23	0.18				0.72	0.25	0.26				0.04	0.11		0.02	0.06
	SW															0.68		0.50
Trachurus japonicus	NE		0.49	0.30			0.38	1.87	0.81	0.03				0.004	0.20	18.63	12.89	7.67
	SW					7.91			7.91						3.75	6.08		4.72
Etrumeus teres	NE		0.93	0.88		31.11	6.61		5.24									
	SW					0.41			0.41		1.02			0.70				
Katsuwonidae	NE					6.56		0.07	0.88						0.01		0.19	0.10
	SW					0.07			0.07		1.43			0.98				
Mene maculata	NE																	
	SW					0.34			0.34		0.15			0.11	12.36	52.38		41.88
Total catch (t)	NE	5,268	8,646	13,913	1,637	810	2,804	2,787	8,038	1,918	1,622	3,602	5,631	12,773	4,862	872	5,325	10,748
	SW					1,498			1,498	806	1,853			2,659	801	2,196		2,997

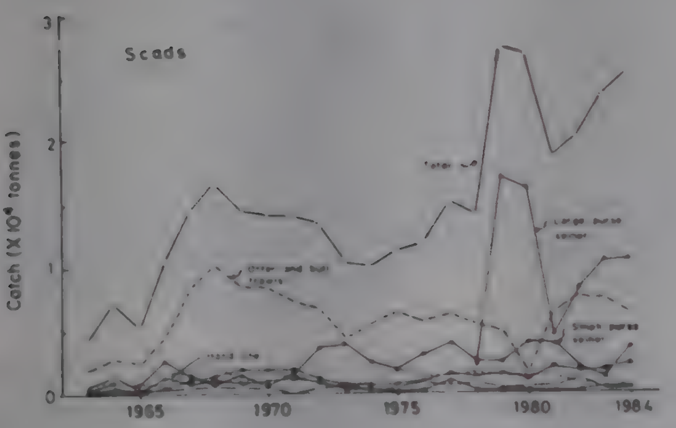

Fig. 1. Annual catches of mackerels of each type of fishery in Taiwan.

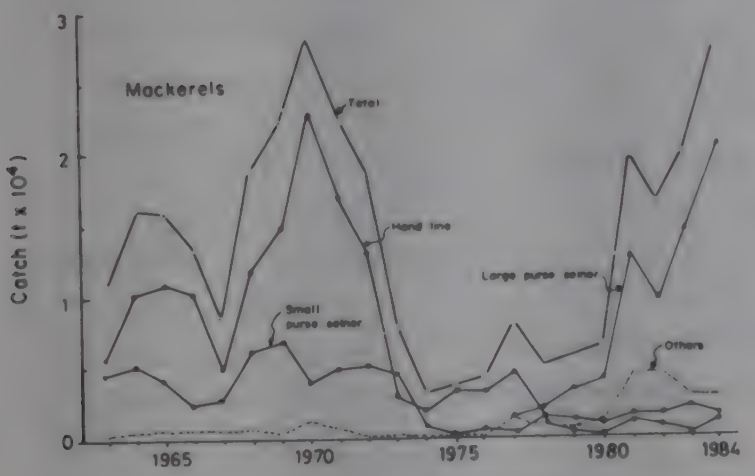

Fig. 2. Annual catches of scads of each type of fishery in Taiwan.

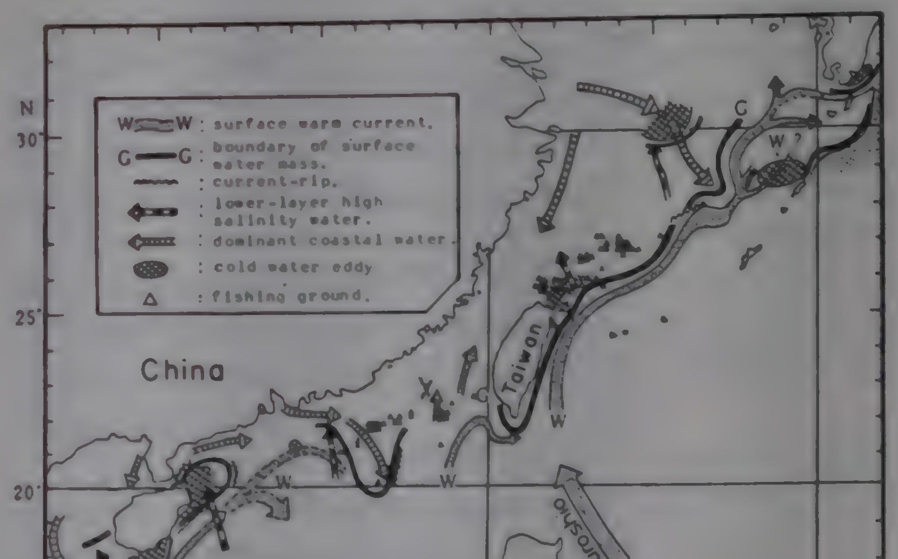

Fig. 3. The ocean current, coastal water and the boundary of water mass in the East and South China Seas and the distribution of the fishing grounds of mackerels and scads in the offshore areas of northeastern and southwestern Taiwan.

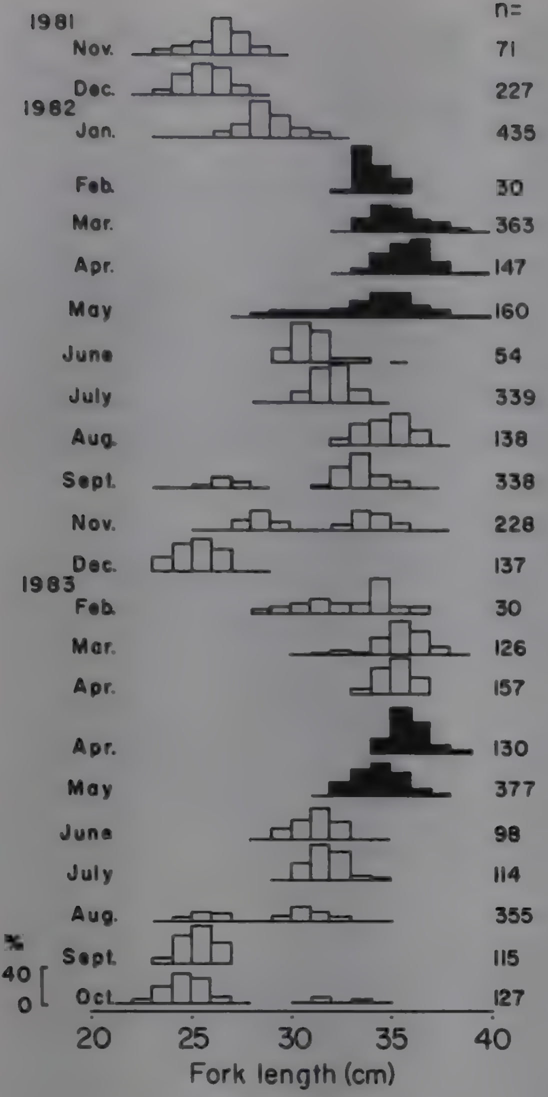

Fig. 4. Monthly length-frequency distribution of *S. australasicus* in the northeastern (open columns) and southwestern (solid columns) offshore areas of Taiwan during the period from November 1981 to October 1983 (N, sample size).

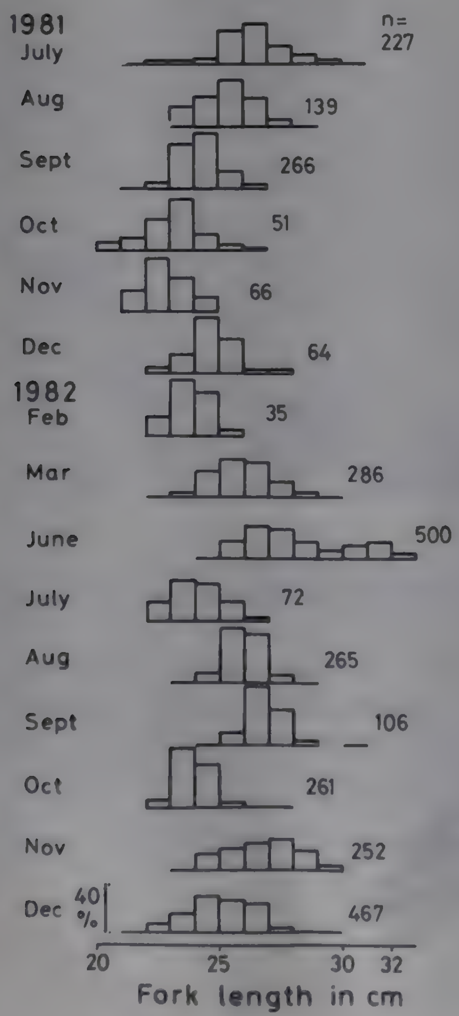

Fig. 5. Monthly length-frequency distribution of *S. japonicus* in the offshore area of northeastern Taiwan, July 1981-December 1982.

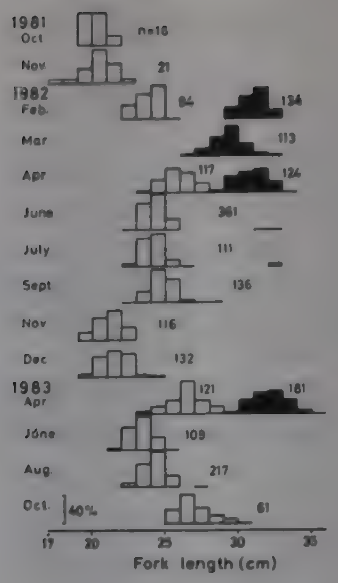

Fig. 6. Monthly length-frequency distribution of *D. akaadsi* in the offshore areas of northeastern (open columns) and southwestern (solid columns) Taiwan, October 1981-October 1983.

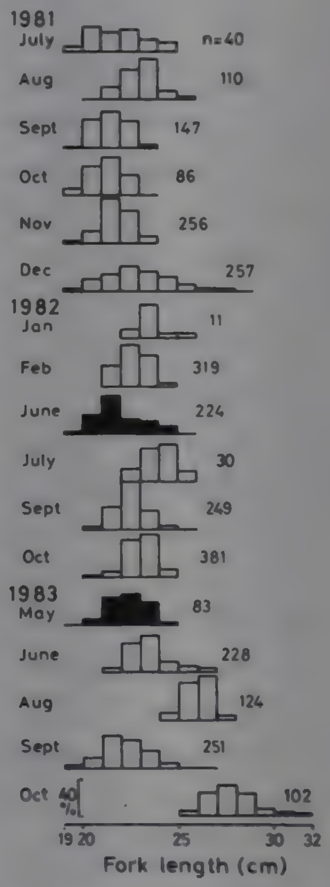

Fig. 7. Monthly length-frequency distribution of *D. macrosoma* in the offshore areas of northeastern (open columns) and southwestern (solid columns) Taiwan, October 1981-October 1983.

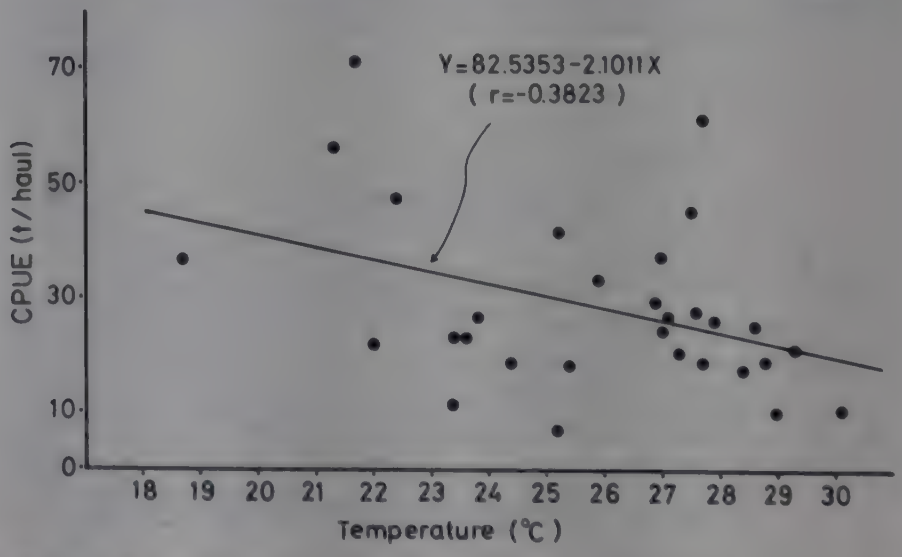

Fig. 8. Relationship between monthly mean CPUEs of *Scomber* spp. and mean water temperature in the offshore area of northeastern Taiwan, July 1982-September 1985.

Aspects of the Biology of a Tropical Cyprinid, *Hampala macrolepidota* (Van Hasselt), with Reference to Food, Feeding Habits and Reproduction

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ABIDIN, A.Z. 1986. Aspects of the biology of a tropical cyprinid, *Hampala macrolepidota* (Van Hasselt), with reference to food feeding habits and reproduction, p. 515-518. *In* J.L. Maclean, L.B. Dizon and L.V. Hosillos (eds.) The First Asian Fisheries Forum. Asian Fisheries Society, Manila, Philippines.

Abstract

A study on some aspects of the biology of *Hampala macrolepidota* (Van Hasselt) with emphasis on its food, feeding habits and reproduction was carried out at Zoo Negara Lake, Kuala Lumpur, Malaysia. The length-weight relationships of male and female adult fish are: $\text{Log}_e W = 3.144\ \text{Log}_e L - 11.9034$ and $\text{Log}_e W = 3.461\ \text{Log}_e L - 13.8770$, respectively. The mouth and its action, dentition, gill rakers, alimentary canal and stomach are described. The gape determines the size of prey eaten. Both juvenile and adult fish have similar gill raker selection. The relative gut indices of the juvenile and adult fish ranged from 1.20 to 1.02 which is typical of carnivorous species. Food analyses of the gut indicate that the food of adult fish is less varied than that of the juveniles and that they feed primarily on crustaceans and fishes. The analyses of the maturity stages, gonadosomatic indices and oocyte diameter patterns show that this fish is able to spawn throughout the year. However, physicochemical and environmental factors determine the actual spawning time. Spawning activity is associated with decreasing temperatures and day-lengths, rising water levels and increasing turbidities. Spawning is protracted and coincides with the rainy season which extends from November to March.

Introduction

Information on the general biology of *Hampala macrolepidota* (Van Hasselt), and in particular its reproductive biology, is scarce, even though the species is a major food and sport fish in South-east Asia. It is also a major component of the fish fauna in the Malaysian freshwater ecosystem (Bishop 1973). This work was carried out to describe some aspects of the biology of this fish. Furthermore, it was anticipated that this study would be useful for the management of large-scale alterations of the aquatic environment, which have severely threatened the fish fauna in most of the water bodies where changes have already occurred.

Materials and Methods

A total of 225 *H. macrolepidota*, consisting of 115 males and 110 females, were caught in a one-day period from December 1978 to November 1979, at fortnightly intervals, from Zoo Negara Lake, Kuala Lumpur, Malaysia. The lake has a surface area of 2.5 ha. The fish were caught at random with cast nets of 5-cm mesh. The total lengths were measured and body and gonad weights were recorded with a triple beam balance (Model Ohaus 4004).

In the laboratory, samples of about ten fishes were selected each month from two successive fortnightly samples. The stomach contents from each fish were removed and preserved. For food analysis, each stomach was cut open, its fullness noted and the contents emptied into a clean petri dish for examination under a dissecting microscope (Model SMZ-2-Nikon). For minute particles, the food items were transferred to clean glass slides and viewed under compound microscope (Model BHC). Each item was identified to the lowest possible taxon.

Before preservation in Bouin's fixative the gonads were first cut into small portions of approximately 5 mm^3 for easy penetration of the fixative. Twenty-four gonads from gravid fish were used for the estimation of fecundity by the gravimetric method (MacGregor 1957) whereby each preserved ovary was weighed individually and the number of eggs from three 10-g subsamples were counted. The means of the total number of eggs in the subsamples were determined. The total number of eggs per ovary was estimated by extrapolation. Regression analysis was done to find the relationship of fecundity to the length and weight (Zar 1974).

Gonads were processed in an automatic duplex processor (Model Shandon Southern CE 0540). They were then embedded in Paraplast R plus, sectioned at a thickness of 6 microns and then stained with Mayer's hematoxylin and alcoholic eosin (Pantin 1969). Gonadosmatic index (GSI) was computed by the formula

$$\text{GSI} = \frac{\text{gonad weight}}{\text{weight of fish} - \text{gonad weight}} \times 100 \quad \text{(Meien 1927).}$$

Ova diameters were measured with a graduated microscope which had been standardized using a micrometer. The number of ova measured per ovary varied between 250 and 300. Monthly means and the range of the ova diameters were recorded. An ocular micrometer was also used in measuring the oocyte diameters.

Results and Discussion

The length-weight relationships of male and female *Hampala macrolepidota* expressed logarithmically are: $\text{Log}_e\text{W} = 3.144\ \text{Log}_e\text{L}-11.903$ and $\text{Log}_e\text{W} = 3.461\ \text{Log}_e\text{L}-13.877$, respectively.

Fish weight tended to increase curvilinearly with length and the exponent b describing the rate of change of weight with length is 3.114 for male fish and 3.461 for female fish.

The condition factors of both male and female fish decreased markedly just before the onset of the spawning season. The condition factor then increased during the wet months, i.e., in November to March coinciding with the spawning period. The condition factors of the female fish were lower during this time as compared to the male fish.

The dentition is composed of fine villiform teeth present on both jaws. The formula for the pharyngeal teeth in *H. macrolepidota* was 5, 3, 1 - 1, 3, 5. Similar results was found by Smith (1945). As in most fishes, the pharyngeal teeth are used in handling material with tough exoskeleton, in particular crustaceans, and to some extent fishes which account for a fair proportion of the diet of this fish. The pharyngeal teeth also kill the prey. Food items are swallowed whole, thus suggesting that the pharyngeal teeth are used for grasping rather than for grinding. The mouth is terminal, large and protractible. The gape is wide, being 20 m^2 in a 150-m^2 fish. The tongue is muscular, unmovable and rather triangular in shape.

The jaw dentition of this fish is quite weak, present in two rows of small conical teeth. Such dentition is of little use in seizing the prey. However, these teeth are important in retaining the prey once in the mouth. Seizure of the prey is achieved primarily as a result of the rapid protrusion of the premaxilla, the prey being sucked into the buccal cavity.

There are four parts of gill arches and all of them bear rakers, two rows on each pair. The gill rakers are small and are of simple conical shape. Gill rakers at the bend of the arches are slightly longer.

Stomach fullness was first observed macroscopically and allocated points according to fullness (Table 1). Fish have full stomachs during the dry season and most gravid females have empty stomachs during the wet months, which correspond to the spawning season.

The food contents of 120 adult individuals and 47 juveniles collected during the study period are presented in Tables 2 and 3. Of the stomachs, 47 or 39.2% were empty. Distended stomachs were mainly observed during the dry season, which is also the nonbreeding period. Most fish stop feeding during the breeding period.

Gut contents were entirely of crustaceans and teleosts. Teleosts consisted mainly of fishes belonging to species of *Osteochilus* and *Tilapia*. *Macrobrachium* made up the crustaceans. Adult fish are entirely carnivorous and slightly piscivorous. Fish bones, scales, mud, sand and debris were the major items found in fish stomachs.

The relative gut index for *H. macrolepidota* is shown in Table 4 which suggests that the adults are carnivorous in their feeding habits while the juveniles are omnivorous on the basis of Odum's (1970) classification of feeding habits. The ratio of the alimentary tract length to the total body length tends to decrease with the increase in fish length.

Oogonia cells occurred singly in all the ovaries. Each oogonium was about 15-20 microns in diameter with a single nucleus and one large nucleolus which stained deeply with hematoxylin.

First growth phase oocytes were larger than oogonia. Primary oocytes were distinguished from oogonia by the presence of distinct chromosomes in various stages of meiotic prophase. First growth phase was subdivided into two definite stages; the chromatin nucleolar stage and the perinucleolar stage.

The first stage after the development of oocytes was the chromatin nucleolar stage. These oocytes had a nucleus with a single conspicuous nucleolus. Chromatin threads were attached to the nucleolus. These oocytes (20-30 microns) were slightly larger than the oogonia.

The perinucleolar stage oocytes were distinguished by the peripheral arrangement of small nucleoli at the inner side of the nuclear membrane. The nucleus was enlarged and the chromosomes lost their distinct nature. Oocytes at the perinucleolar stage had diameters of 30-90 microns. The cytoplasm of oocytes in this stage strained deeply with hematoxylin.

Second growth phase oocytes was characterized by the formation and accumulation of yolk. The larger yolk vesicles were about 12 microns while the smaller granules were about 1 micron in diameter. These two types of yolk were formed sequentially, with yolk vesicles preceding yolk granules.

The first indication of yolk formation was the presence of vesicles in the periphery of the oocytes' cytoplasm. Oocytes at this stage measured about 150 microns and the vesicles about 12 microns in diameter. Initially, each vesicle was formed as a minute body, but later they increased in both size and number until they occupied the whole cytoplasm. The yolk vesicles appeared as vacuoles when sections were stained with hematoxylin and eosin. At this stage of oocyte development, the follicular layer was fairly well developed. The zone radiata started to develop during this stage.

The yolk granule stage (360-380 microns) was the final stage of vitellogenesis and oocyte development. Yolk granules formed only in oocytes with fully developed yolk vesicles. The granules first developed close to the zone radiata. They were not very distinct when stained with hematoxylin and eosin. Ripe stage oocytes measured about 800-850 microns in diameter.

Seasonal changes in GSI support the conclusion that March is the month when most fish start to breed. There was a positive correlation ($r = 0.4584$; $P > 0.005$) between the GSI and rainfall, the gonads being heavier when rainfall is more abundant. Two peaks were observed in a one-year cycle and both peaks coincided with the rainy season: that in August coincided with the second rainy season brought about by the southwest monsoon.

The means and ranges of oocyte diameters for each month indicate the presence of spawning individuals throughout the year.

Of the 202 adult specimens caught, 102 were males and 100 were females, the overall ratio being not significantly different (x^2 test) from the hypothetical 1:1.

From 24 specimens with total lengths ranging from 205 to 373 mm and weights ranging from 97.2 to 750.0 g, the number of eggs per female ranged from 7,132 to 62,031, with a mean of 29,495 eggs. The results for *H. macrolepidota* are similar to those of Tandipayuk and Pandang (1982) who found that the fecundity of fishes 213-422 mm in fork length and 187-1,752 g in body weight was 9,743-47,812 eggs, with a mean of 24,558.

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Table 1. Number of fish in each fullness category and mean fullness per month.

Fullness (points)	Number of fish in each category per month											
	J	F	M	A	M	J	J	A	S	O	N	D
0	11	12	15	11	—	—	—	6	7	3	5	10
½	4	5	10	8	2	—	—	1	2	5	5	2
1	2	1	6	9	5	—	—	—	4	3	4	1
2	—	—	—	3	2	2	5	—	2	—	1	1
3	—	—	—	4	2	2	3	—	1	—	—	—
4	—	—	—	4	3	2	2	—	—	—	—	—
5	—	—	—	1	1	—	2	—	—	—	—	—
Fish	17	18	31	40	15	6	12	7	16	11	15	14
Total points	4	3½	11	52	37	18	37	½	12	5½	8½	4
Mean	0.2	0.2	0.4	1.4	2.5	3.0	3.1	0.1	0.8	0.5	0.6	0.3

Table 2. Diet composition for all juvenile *Hampala macrolepidota* as determined by percentage occurrence and points method (no. examined – 47; no. with food – 47).

Food category	Occurrence	Points
Plankton		
(Phytoplankton)		
Microcystis	42.6	2.4
Selenastrum	53.2	1.8
Melosira	29.8	1.0
Anabaena	85.1	5.2
Coelastrum	25.5	0.8
Oscillatoria	48.9	1.6
Cosmarium	38.3	0.8
Gleocystis	74.4	1.0
Glosterium	21.3	1.5
(Zooplankton)		
Copepods	89.4	2.0
Cladocera	53.2	3.5
Crustaceans		
Macrobrachium sp.	95.7	55.0
Teleostei		
Tilapia sp.	87.2	60.0
Osteochilus sp.	68.1	30.0
Fish scales	95.7	20.0
Fish bones	80.9	10.0
Plant materials		
(Highly digested)	63.8	25.0

Sand particles are found in about 60% of the stomachs examined.

Table 3. Diet composition for all adult *Hampala macrolepidota* as determined by percentage occurrence and points method (no. examined – 120; no. with food – 73).

Food category	Occurrence	Points
Crustaceans		
Macrobrachium sp.	65.8	25.0
Teleostei		
Tilapia sp.	72.6	40.0
Osteochilus sp.	68.5	32.0
Fish scales	100.0	5.0

Debris and sand particles are found in about 10% of the stomachs.

Table 4. Relative gut index (RGI) of *Hampala macrolepidota.*

Size of fish (mm)	RGI (Range)
Up to 60 mm	1.20 (1.05 – 1.30)
60-120 mm	1.12 (1.02 – 1.25)
Above 120 mm	1.02 (1.00 – 1.08)

Combinations of Dietary Fat Sources in Dry Diets for *Chanos chanos* Fingerlings

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Abstract

A study was conducted to determine the effects of 1:1 ratio of several dietary fat sources added in semipurified diets at 10% level on milkfish fingerlings. Results showed that the cod liver oil + coconut oil diet promoted significantly the highest growth rate. However, beef tallow + coconut oil and pork lard + coconut oil also gave good growth, feed conversion and survival. The hepatosomatic index of milkfish did not differ significantly among treatments. Proximate analysis of whole milkfish body showed that as body fat increased, body protein, ash and moisture levels decreased. Weight gains were positively correlated with body fat. Also, the various groups of fatty acids in diets and in milkfish have positive correlations. Diets containing high levels of saturated fatty acids resulted in low levels of milkfish polyunsaturated fatty acids.

Introduction

The formulation of artificial diets depends upon the economical energy provided by fats as dietary fats save protein in the diet from being used as an energy source. Fish oil serves as an excellent source of $\omega 3$ fatty acids, whereas soybean oil and corn oil contain high amounts of $\omega 6$ fatty acids. Although coconut oil and animal fat are low in both $\omega 3$ and $\omega 6$ fatty acids and are more saturated than fish or vegetable oils, these are also good sources of energy.

In this study, various combinations of dietary fat sources were incorporated in semi-purified diets. The objective was to test these dietary fat sources for growth, feed conversion, hepatosomatic index, survival, proximate and fatty acid composition of milkfish fingerlings.

Materials and Methods

Milkfish fingerlings were obtained from a nursery pond and acclimated to laboratory conditions for ten days before the start of the experiment. During this period, the fish were fed with the control diet (Table 1). Twenty fish (mean weight 0.95 g; mean total length 52 mm) selected at random, were stocked in each of the 60-l oval fiberglass tanks containing 50 l of seawater. Each tank was provided with continuous aeration and flow-through seawater (0.5 to 1.0 l/min.). The average water temperature was 28°C and the salinity was 33 ppt.

A completely randomized design with three replications per treatment was followed. Each of the eleven experimental diets was fed at a rate of 10% of the biomass per tank daily. Every morning, feces were siphoned out. Feed allowances were adjusted after biweekly sampling.

Ten isocaloric (340 kcal/100 g diet) and isonitrogenous (40% crude protein) semipurified diets containing 1:1 ratios of various dietary fat supplements at 10% level were prepared (Tables 1 and 2). Table 3 shows the percentage fatty acid composition of the dietary lipids. The control was a fat-free diet made isocaloric to the other diets by the substitution of dextrin. Metabolizable energy was estimated based on fish physiological values (Brett and Groves 1979). After thorough mixing, each diet was pelleted, oven dried at 60°C, crushed to obtain 425 μm particle size and kept refrigerated at -5 to 0°C.

After eight weeks of feeding, whole fish samples were collected for proximate analysis. Moisture was determined by evaporation in the oven at 100°C until constant weight was obtained. Total nitrogen was analyzed with the Kjeltec Auto System and converted into total protein by 6.25 as factor. Crude fat was determined by Soxtec extraction with anhydrous diethyl ether. Ash content was determined from the residue remaining after incineration of samples at 550°C in a muffle furnace. The total lipid of whole milkfish body was extracted and purified by the method of Bligh and Dyer (1959). The fatty acids of total lipids were esterified by the saponification-transesterification method of Metcalfe et al. (1966).

A Shimadzu GC-4C Gas Chromatograph equipped with a flame ionization detector was used to analyze the fatty acid methyl esters (FAME). The column was of stainless steel, 2 m long, 3 mm I.D. and packed with 10% di-ethylene glycol succinate (DEGS) on a 120-mesh

Chromosorb support, operated isothermally at 180°C with 30 ml/min. flow of nitrogen. FAME was identified and quantified with an electronic integrator Hewlett Packard 3390 A. Authentic standards and literature for published oils were used for identification of FAME.

Results and Discussion

The dietary fat sources influenced the growth and feed conversion ratios of milkfish fingerlings. As shown in Fig. 1 and Table 4, the diet containing cod liver oil + coconut oil promoted significantly the highest growth among the diets tested. Pork lard + coconut oil and beef tallow + coconut oil diets also promoted high growth rates. The growth rate of milkfish fed a fat-free diet was significantly the poorest, although no physical signs of fatty acid deficiency were observed. The efficiency of the diets as expressed in feed conversion ratios reflected the growth rates of milkfish fingerlings.

The ω3 fatty acids are known to be more effective as essential fatty acids for *Salmo gairdneri* (Lee et al. 1967; Castell et al. 1972) whereas ω6 fatty acids are effective for *Tilapia zillii* (Kanazawa et al. 1980) and *Oreochromis niloticus* (Teshima et al. 1982; Takeuchi et al. 1983). However, *Cyprinus carpio* (Watanabe et al. 1975) and *Anguilla japonica* (Takeuchi et al. 1980) seem to require not only 18:3 ω3 but also 18:2 ω6 for their good growth. *Ictalurus punctatus* appears to have much lower essential fatty acid requirements (Stickney and Andrews 1971, 1972; Murray et al. 1977). Although milkfish had the highest growth from cod liver oil + coconut oil diet with an ω3/ω6 ratio of 5.5, the good performance of milkfish fed beef tallow + coconut oil and pork lard + coconut oil diets showed that its requirement for essential fatty acids was not very exacting.

In all treatments, survival ranged from 90 to 100%. There was no indication of toxic effects being produced by any diet since the survival rates were high. No pathological abnormalities were observed among the fish during the feeding trial.

High relative liver weights and discolored livers often give an indication of unfavorable feed. This effect is often associated with the dietary fat. In this study, these traits in livers of milkfish fingerlings were not significantly different. The hepatosomatic index (liver weight x 100/body weight) did not differ significantly among treatments (Table 4).

The proximate composition of milkfish fed various fat sources is presented in Table 5. The presence of large amounts of fat compared to the initial content means that fat rather than carbohydrates is the favored energy reserve for milkfish. The fish expend little energy for the capture of food, a condition which is conducive to deposition of fat. As the body fat of milkfish increased, moisture, protein and ash levels decreased, with correlation coefficients of r = -0.82, r = -0.87 and r = -0.61, respectively. The significant inverse correlation between body fat and moisture contents demonstrated the diluting effect of fat on moisture levels.

Correlation analysis of weight gain data and body composition showed that rapidly growing fish had decreased body protein (r = -0.68) and moisture (r = -0.68) levels. A significant positive correlation existed between weight gain and body fat (r = 0.80). This indicated that the more rapidly growing fish were fatter.

The composition of fatty acid deposition of milkfish (Table 6) reflected that of dietary fat (Table 3). The positive correlations between the fatty acid composition of diets and milkfish for the various groups of fatty acids are as follows: ω3, r = 0.66; ω6, r = 0.76; PUFA, r = 0.85; saturated, r = 0.80 and ω3/ω6 ratio, r = 0.93. Diets containing high levels of saturated fatty acids resulted in low levels of milkfish polyunsaturated fatty acids (r = -0.82). Although most diets contain high saturated fatty acid contents, the saturated fatty acids of milkfish did not exceed 48% level. Like trout (Yu et al. 1977) and channel catfish (Stickney and Andrews 1971), milkfish appears to have a mechanism that regulates and maintains a proper level of lipid saturation.

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Table 1. Composition of milkfish diets.

Composition	Per cent in the diet 1	 2-11
Casein	20.00	20.00
Gelatin	20.00	20.00
Dextrin	48.50	24.30
Fat[a]	0.00	10.00
Vitamin mix[b]	3.00	3.00
Mineral mix[c]	4.00	4.00
B.H.T.	0.05	0.05
C.M.C. (Gelatinized)	3.00	3.00
α-cellulose	1.45	10.95

[a] See Table 2.

[b] Vitamin mix (mg/100 g diet): p-amino benzoic acid, 72.24; Biotin, 1.09; Inositol, 726.34; Nicotinic acid, 145.06; Ca-pantothenate, 50.78; Pyridoxine HC1, 8.64; Riboflavin, 36.26; Thiamine HC1, 10.88; Menadione, 8.64; Vit. A, 18.14; α-tocopherol, 72.55; Cyanocobalamine, 0.02; Calciferol 1.82; Choline chloride, 1482.65; Ascorbic acid, 362.66; Folic Acid, 2.72; α-cellulose, 0.51.

[c] Mineral mix (mg/kg diet): NaC1, 147.04; $MgSO_4$, 548.00; $Na_2HPO_4.7H_2O$, 348.80; K_2HPO_4, 929.20, $Ca(H_2PO_4)_2.H_2O$, 543.20; $FeC_6H_5O_7$, 118.80, Ca-lactate, 1308.00, $AlC1_3.6H_2O$, 0.72, $ZnSO_4.7H_2O$, 14.32; $CuC1_2.2H_2O$; 0.40; $MnSO_4.H_2O$; 3.20; KI, 0.64; $CoCl_2.6H_2O$; α-cellulose, 3.68.

Table 2. Composition of dietary fat.

Diet	Per cent dietary fat		
1	No fat added		
2	5% cod liver oil	+	5% coconut oil
3	5% cod liver oil	+	5% soybean oil
4	5% cod liver oil	+	5% corn oil
5	5% cod liver oil	+	5% beef tallow
6	5% beef tallow	+	5% coconut oil
7	5% beef tallow	+	5% soybean oil
8	5% beef tallow	+	5% corn oil
9	5% pork lard	+	5% coconut oil
10	5% pork lard	+	5% soybean oil
11	5% pork lard	+	5% corn oil

Table 3. Percentage fatty acid composition of dietary lipids

Fatty acid	Diet 2	 3	 4	 5	 6	 7	 8	 9	 10	 11
12:0	27.5	–	–	–	20.3	–	–	55.5	–	–
14:0	13.1	2.9	2.3	3.6	11.3	1.0	2.5	4.9	1.7	1.6
16:0	12.5	13.2	14.4	26.3	17.7	22.3	14.3	10.7	18.8	19.6
16:1ω7	6.4	7.1	6.3	3.7	1.7	0.7	1.2	–	–	–
18:0	1.7	1.9	1.8	32.2	29.7	11.5	27.1	17.6	30.7	31.9
18:1ω9	17.0	24.2	30.1	16.3	16.8	28.4	12.0	6.8	32.2	32.4
18:2ω6	2.2	32.8	25.4	2.4	2.8	32.2	38.4	3.0	15.4	13.8
18:3ω3	1.1	3.1	3.6	1.1	–	3.9	6.5	1.5	1.2	0.8
20:1ω9	7.3	5.6	6.3	6.2	–	–	–	–	–	–
20:5ω3	9.5	8.2	8.4	6.5	–	–	–	–	–	–
22:1ω9	0.2	0.1	0.2	0.4	–	–	–	–	–	–
22:6ω3	1.5	1.1	1.2	1.3	–	–	–	–	–	–
ω3	12.1	12.4	13.2	8.9	–	3.9	6.5	1.5	1.2	0.8
ω6	2.2	32.8	25.4	2.4	2.8	32.2	38.4	3.0	15.4	13.8
PUFA	14.3	45.0	38.6	11.3	2.8	36.1	42.9	4.5	21.1	36.7
Saturated	54.8	18.0	18.5	62.1	79.0	34.8	43.9	88.7	61.2	63.0
ω3/ω6	5.5	0.4	0.5	3.7	–	0.1	0.2	0.5	0.1	0.1

Table 4. Growth rate, weight gain, hepatosomatic index (HI), feed conversion ratio (FCR) and survival rate of milkfish fingerlings

Treatment	Growth rate (%)	Weight gain (g)	HI (%)	FCR	Survival rate (%)
1	213.68^e	2.03^e	1.92	3.48^e	90
2	576.68^a	5.46^a	1.12	1.22^a	100
3	345.26^c	3.28^c	1.28	2.35^c	100
4	355.79^c	3.38^c	1.18	2.42^c	100
5	385.26^c	3.66^c	1.18	2.40^c	100
6	450.53^b	4.28^b	1.78	1.90^b	100
7	306.31^d	2.91^d	1.45	2.98^c	98
8	345.26^c	3.28^c	1.28	2.50^c	97
9	457.89^b	4.35^b	1.21	1.86^b	100
10	297.89^d	2.83^d	1.25	2.61^c	98
11	347.37^c	3.30^c	1.06	2.33^c	98

Treatment means with the same superscripts are not significantly different at $P > 0.05$.

Table 5. Moisture and dry matter percentages of crude protein, crude fat and ash contents of milkfish fingerlings.

Treatment	Moisture	Percentages Crude protein	 Crude fat	 Ash
Initial	81.64	70.90	3.64	23.05
1	75.45^a	71.23^a	13.12^e	14.06^b
2	72.88^c	65.67^b	20.77^b	12.68^g
3	74.78^b	67.71^b	16.86^e	14.64^c
4	74.80^b	66.84^b	17.56^d	15.77^a
5	73.09^{bc}	66.81^b	19.87^c	12.61^g
6	74.56^b	66.53^b	19.64^c	13.23^f
7	74.41^b	67.58^b	18.42^d	13.30^f
8	74.15^b	67.28^b	17.63^d	14.20^d
9	72.68^c	66.59^b	21.77^a	11.89^h
10	74.79^b	66.08^b	18.39^d	14.96^b
11	72.88^c	66.01^b	19.66^c	13.63^e

Treatment means with the same superscripts are not significantly different at $P > 0.05$

Table 6. Percentage fatty acid composition of milkfish lipids

Fatty acid	Diet 1	 2	 3	 4	 5	 6	 7	 8	 9	 10	 11
12:0	0.3	20.0	0.3	0.2	0.3	11.2	0.2	0.1	11.2	0.2	0.2
14:0	1.0	11.1	2.7	2.2	2.9	11.4	1.7	1.2	11.1	1.5	1.0
16:0	29.0	14.3	17.8	14.6	23.5	22.7	23.3	20.1	22.0	23.9	20.5
16:1ω7	18.1	15.8	14.1	14.2	20.0	21.8	21.4	21.4	15.2	10.5	10.6
18:0	6.1	2.2	2.2	2.5	2.8	2.7	2.2	2.6	2.8	2.5	2.9
18:1ω9	32.5	17.4	22.1	23.8	30.1	21.4	23.0	24.6	21.4	24.5	26.2
18:2ω6	6.2	3.8	17.3	18.6	3.7	2.5	15.2	16.5	6.2	19.6	20.9
18:3ω6	0.3	–	4.0	3.4	–	–	3.8	3.3	–	4.1	3.5
18:3ω3	1.2	4.6	5.1	5.3	5.7	1.1	1.4	1.5	0.8	1.2	1.6
20:1ω9	1.7	1.4	1.2	0.9	0.4	0.9	0.8	0.6	1.0	0.9	0.7
20:3ω6	1.2	0.5	0.8	0.8	1.1	1.5	1.5	1.3	1.1	1.2	1.0
20:4ω6	0.8	1.7	3.0	2.5	1.7	0.2	1.4	0.9	1.1	2.4	1.8
20:4ω3	0.4	0.8	2.9	2.3	0.9	0.5	2.5	1.9	1.3	3.4	2.7
20:5ω3	0.3	1.9	1.9	2.7	2.0	0.2	0.1	0.9	2.1	1.9	2.8
22:4ω6	0.3	0.2	0.2	0.7	0.2	0.2	0.2	0.7	0.5	0.5	1.0
22:4ω3	0.2	0.4	0.3	0.3	0.3	0.2	0.1	0.1	0.4	0.3	0.2
22:5ω3	0.2	1.4	1.6	2.1	1.5	0.4	0.6	1.1	0.7	0.8	1.4
22:6ω3	0.2	2.8	2.5	3.1	2.9	1.1	0.6	1.2	1.1	0.6	1.3
ω3	2.5	11.9	14.3	15.8	13.3	3.5	5.3	6.7	6.4	8.2	9.8
ω6	8.8	6.2	25.3	25.8	6.7	4.4	22.1	22.7	8.9	27.8	28.2
PUFA	11.3	18.1	39.6	41.6	20.0	7.9	27.4	29.4	15.3	38.0	38.0
Saturated	36.4	47.6	23.0	19.5	29.5	48.0	27.4	24.0	47.1	28.1	24.6
ω3/ω6	0.3	1.9	0.6	0.6	2.0	0.8	0.2	0.3	0.7	0.3	0.3

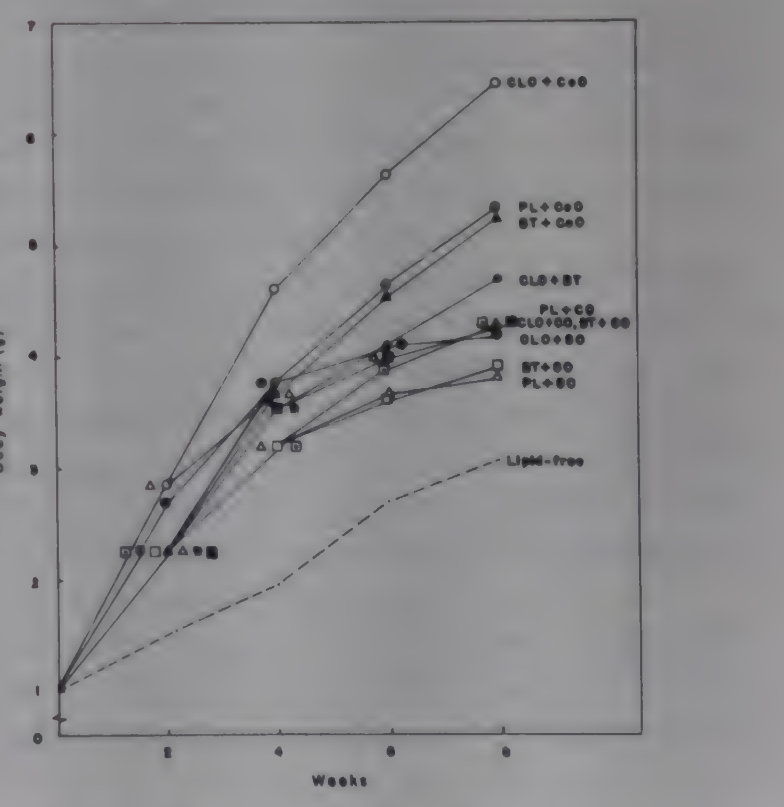

Fig. 1. Mean body weight of milkfish fingerlings fed various diets. BT = beef; CLO = cod liver oil; CoO = coconut oil; CO = corn oil; PL = pork lard; SO = soybean oil.

Azolla pinnata as a Dietary Component for Nile Tilapia, *Oreochromis niloticus**

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Abstract

Nile tilapia *(Oreochromis niloticus)* fingerlings were fed *Azolla pinnata* (Bangkok strain) in varying forms and states and at varying percentages in formulated feeds. A standardized 28-day assay was used. Fed on fresh *Azolla* alone, fingerlings lost 24-28% body weight. With solar-dried pellets and powder form, tilapia also lost weight although fed *ad libitum*. Although pellets with 75% dried *Azolla* generally enabled the fish to grow positively, growth rates were still significantly lower than those for the control diet mix of marine fishmeal, rice bran, corn starch, corn meal and micronutrient premix. Results indicate lowered growth performance and worsening FCRs with increased dried *Azolla* incorporation in the diet. Likewise, adult males fed *ad libitum* with fresh *Azolla* and dried pellets had growth rates lower than those fed the control diet. The *Azolla pinnata* used in this study was deficient as a feed for Nile tilapia. However, further studies still have to be made on the possible reasons. Ways to improve the nutritional value of such diets are discussed.

Introduction

Nile tilapia *(Oreochromis niloticus)* is the most important tilapia cultured in the tropics and there is need for low cost feeds to increase its production in culture systems. Jauncey and Ross (1982) have summarized the available information on tilapia feeds.

The aquatic fern, *Azolla*, fixes atmospheric nitrogen through its symbiotic relationship with the blue-green alga *Anabaena azollae*. *Azolla* has attracted attention as a nitrogenous fertilizer (Peters et al. 1982) and as a source of dietary nitrogen for herbivorous fish and livestock (Subudhi and Singh 1978; Edwards 1980; Lumpkin and Plucknett 1980). It has been assumed that feeding fresh *Azolla* to *O. niloticus* and other tilapias in fishponds and in integrated rice-fish culture is beneficial. Its popularity has stirred a rush among fish farmers for its propagation and use for fish culture.

This study assessed the use of fresh and dried *Azolla* as feeds for *O. niloticus* fingerlings and adults, including the possible replacement of a fish meal-based pelleted diet with diets containing *Azolla*.

Materials and Methods

An inoculum of *Azolla pinnata* (Bangkok strain), obtained from the International Rice Research Institute (IRRI), Los Baños, Philippines, was initially cultured in a nutrient solution (Ramirez et al. 1979) for propagation. The plant was subsequently stocked into 1.5 m diameter, 0.5 m deep outdoor plastic pools where it was cultured in fertilized water over a thin soil layer.

Proximate analyses of sun-dried *Azolla* and other experimental diets were performed by the Department of Chemistry, University of the Philippines in Los Baños, following standard feed analysis procedures (Table 1).

The Institute of Aquaculture, University of Stirling, Scotland, also carried out a proximate analysis of *Azolla* (Table 2) and determined the essential amino acid profile of a freeze-dried sample of the plant (Table 3).

Nutritional assays for fingerlings were conducted in aerated 68-1 aquaria, 20 fingerlings/aquarium. Test diets were assigned at random to duplicate or triplicate aquaria.

In all cases, fingerlings were first acclimated to assigned experimental feeds prior to the start of actual feeding experiment which ran for 28 days. Daily rations were fed at 9 a.m. and 5 p.m. Feeding levels were adjusted following weekly weighing and measurements of all fish. Daily cleaning of aquaria and water exchange minimized the development of extraneous feeds. Surface water temperature ranged from 24 to 30°C.

Assays on adult males were performed in 225-1 aquaria each stocked with 10 fish. Routine care of the fish and conduct of the experiment, which ran for 30 days, followed those for fingerlings. Surface water temperature ranged from 27 to 31°C.

Results were evaluated with standard parameters. Daily specific growth, G%, feed conversion ratio, FCR, and essential amino acid index, were calculated with formulae developed by Jauncey and Ross (1982). Statistical analyses followed those described by Steel and Torrie (1960).

Fresh *Azolla* was fed at 10% and 20% body weight/day and excess (some always present) levels using triplicate aquaria. Daily rations of the plant were removed from cultures, flushed with water to remove extraneous materials, drained and weighed. Adjustments to the rations were made following weekly weighings of all fish.

Table 1 lists the test diets used with dried *Azolla*. Pure dried *Azolla*, prepared in powdered (diets 1 and 2) or pelleted (diets 3 and 4) forms, was assessed alongside other pelleted experimental feeds (diets 5 to 9) which include dried *Azolla* at varying levels, 90%, 75%, 50%, 25% and 10% of the control (diet 10). This reduced the proportions of the complete mix used in the control except for Afsillin which was kept constant at 1%. The control is pelleted diet used in routine feeding at the Institute of Fisheries Development and Research, University of the Philippines in the Visayas.

All diets were supplied to the fingerlings at the rate of 5% body weight/day except for diet 2 which was given in excess and diet 4 given at 10% body weight/day. All diets, including the pure dried *Azolla* were evaluated against the *Azolla*-free control and against each other. Fingerlings were acclimated to the diets in the aquaria for three days before the experiment.

Adult male *O. niloticus* received three feeds: fresh *Azolla*, dried *Azolla* pellets and the control diet. The fish were fed the test diets *ad libitum* except for the control which fed at the rate of 5% body weight/day, adjusted weekly.

Composition and preparation of the dry feeds were identical to those for fingerlings and their respective proximate analyses were presumed to approach closely the analyses in Table 1.

Results

All feeds, including fresh and dried *Azolla*, were readily accepted by the fish. Daily rations were normally consumed a few minutes after they were given, except where feeding was intentionally at excess levels.

All the fish fed fresh *Azolla* suffered loss of weight (Table 4). There were no significant differences in weight loss and survival between different feeding rates. When offered *Azolla* in excess (40% body weight/day), fish consumed about 20% body weight/day.

Results of the nine test diets with dried *Azolla* and the control are summarized in Table 5. Negative growth rates resulted from treatments 1, 3 and 4. Treatments 2, 5 and 6 gave positive but unacceptably slow growth. The control diet resulted in better growth than all levels of *Azolla* incorporation up to and including 50% (treatment 7). Despite high variability, the results indicate a trend in lowered growth performance and worsening FCRs with increasing *Azolla* incorporation. The FCR for treatment 2 was very poor due to excess feeding.

Azolla, whether fresh or in dried pelleted form, caused loss of weight of adult males, despite feeding to satiation (Table 6). Negative growth from these diets affirmed similar results with fingerlings. The adult fish on the control diet grew more slowly than the fingerlings. The FCR (5.18) was also poor, suggesting that 5% body weight/day feeding rate was not efficient for the size of fish. Jauncey and Ross (1982) citing Macintosh and De Silva (in press), conclude that by the time tilapia have grown to 50 g they will not generally accept feed more than 3% of their body weight daily.

Discussion

This study reveals the inadequacy of an all-*Azolla pinnata* (Bangkok strain) diet for *O. niloticus*. The high water content of the fresh *A. pinnata* (average 94%) may be a deleterious factor, but body weights were not maintained on a diet of fresh *Azolla* even when an excess was provided. Moreover, dried *Azolla* with a water content of about 11%, was of negligible nutritional value, as growth experiments confirmed. Poor growth responses to dried all-*Azolla* diets were obtained whether they were fed as powder or as pellets.

The proximate analyses of *Azolla* (Tables 1 and 2) indicate its nutrient deficiencies. First, the protein content of the high-*Azolla* diets was below accepted values for tilapia nutrition which range from 20% to 30%. But for the low protein level of the *Azolla* used, its essential amino acid (EAA) profile appears favorable as a tilapia feed. Table 3 compares its EAA levels with the quantitative requirements of *O. mossambicus* as reported by Jauncey et al. (1983). The requirements of *O. niloticus* are likely to be similar. The EAA index of *Azolla* protein was calculated to be 71.75%. The three EAAs most frequently limiting in plant protein, lysine, methionine and phenylalanine, were present in especially high quantities. However, this *Azolla pinnata* strain was limiting in tryptophan and slightly deficient in threonine. Hence, it is suggested that *O. niloticus* fed here all-*Azolla* or high-*Azolla* diets suffered from deficiencies in these two EAAs. However, the biological availability of the various amino acids was not determined. The results suggest that an *Azolla* species/strain with a higher protein content would have to be developed to utilize its favorable EAA profile in fish feed formulation.

The all-*Azolla* and high-*Azolla* diets also had low lipid values. In these diets, a substantial portion of the protein supplied by the plant was conceivably used for energy rather than for growth. Jauncey and Ross (1982) suggest a dietary lipid level of 6-10% for fish of the size used in this study to allow maximum utilization of protein for growth.

Further, certain fatty acids, notably polyunsaturated fatty acids of the $\omega 6$ series are known to be essential to fish. The efficiency of the *Azolla* diets used may have been limited by inadequate quantities of essential fatty acids. These were not quantified in this study.

Tables 1 and 2 disagree on the size of the unavailable fraction of carbohydrates. Table 1 lists the crude fiber content of dried *Azolla* and high-*Azolla* preparations as about 7% of dry matter. Table 2, on the other hand, shows that the difference between total and available carbohydrates would give a fiber content of about 53% dry matter. The discrepancy between the two estimates cannot be accounted for. However, other published data show similar problems (Singh and Subudhi 1978; Subudhi and Singh 1978). If the true level of indigestible fibrous compounds is indeed very high, then this could adversely affect the nutritional value of *Azolla* for *O. niloticus*. High percentages of indigestible components have been cited as causes for poor digestibility and lowered availability of the energy content of plant feeds as well as reductions in fish growth, feed conversion efficiency, net protein utilization, condition and carcass fat (Buddington 1979; Appler and Jauncey 1983; Anderson et al. 1984).

Based on assumed digestibilities of 80% for protein, 90% for lipid, and 60% for available carbohydrate, the likely digestible energy of dried *Azolla* was estimated to be 1.05 kcal/g. According to Jauncey (pers. comm.), this digestible energy level is too low even for a supplementary feed.

This *Azolla* contained only half the level of phosphorus required for tilapia feeds, a deficiency probably worsened by the high calcium content, which can reduce availability of dietary phosphorus (Jauncey, pers. comm.). High calcium levels can also inhibit the assimilation of essential dietary trace elements such as zinc. The high calcium levels reflect the high ash content which is in itself a negative factor. No analyses were made for other minerals.

Dietary protein and lipid levels, growth rate, feed conversion and degree of *Azolla* inclusion in the control were apparently correlated. The fish meal control diet contained near-optimum levels of protein and lipid for tilapia and its efficiency was confirmed by the assays. Feeds where *Azolla* replaced only 10% to 25% of the control formulation probably also met the requirements of *O. niloticus* fingerlings for protein and lipid. The adult fish also grew well on the control feed and would probably also respond favorably to low-*Azolla* feeds, as did the fingerlings. Larger fish generally tend to have lower qualitative nutrient requirements, but the results here show that *O. niloticus* adults did not utilize the all-*Azolla* diets any more efficiently than did the fingerlings.

On the evidence presented, inclusion of dried *Azolla* in *O. niloticus* diets at any level may not be recommended. However, supplementation may make inclusion possible. *A. pinnata* with a higher protein content can be obtained and deficiencies in the essential amino acids, tryptophan and threonine, could be supplied from a variety of sources. However, the availability of other essential animo acids, particularly those that are commonly limiting, must be established. The low energy content of *Azolla* and its possible limitations in essential fatty acids may be remedied by providing alternative energy sources. Increasing its energy content would then spare the proteinaceous component for fish growth.

Additional work on *Azolla* as a fish feed component should include determination of the digestibilities of its energy and protein constituents and a more accurate determination of its fiber content or measurement of specific components such as the celluloses. Finally, it would be worthwhile to replicate this work with the macrophyte-feeding tilapias *T. rendalii* and *T. zillii* which may be better adapted to deal with fibrous plant materials than *O. niloticus*.

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Table 1. Composition (g/100 g) and proximate analyses (%) of test diets offered to *Oreochromis niloticus* fingerlings.

	Treatment/diet no.									
	1	2	3	4	5	6	7	8	9	10 (control)
Dried *Azolla** powder	100	100	95	95	90	75	50	25	10	–
Fish meal	–	–	–	–	4	10	20	30	36	40
Rice bran	–	–	–	–	4	10	20	30	36	40
Corn meal	–	–	–	–	1	2.5	5	7.5	9	10
Corn starch	–	–	5	5	0.9	2.25	4.5	6.75	8.1	9
Afsillin**	–	–	1	1	1	1	1	1	1	1
Moisture (%)	11.21	11.21	10.14	10.14	9.89	10.74	13.82	14.58	14.26	11.92
Ash (%)	33.46	33.46	27.91	27.91	27.81	27.46	19.91	14.89	12.98	10.14
Crude fat (%)	3.30	3.30	3.38	3.38	2.89	3.22	5.41	7.31	7.45	12.41
Crude protein (%)	15.38	15.38	14.34	14.34	13.71	15.71	20.78	21.58	23.32	33 37
Crude fiber (%)	7.16	7.16	7.63	7.63	7.52	6.98	4.54	4.26	3.24	4.57
Nitrogen-free extract (%)	29.49	29.49	36.60	36.60	38.18	35.89	35.54	37.38	36.75	27.09

**Azolla pinnata* (Bangkok strain).

**(Micronutrient premix: E.R. Squibb and Sons, Manila).

Table 2. Proximate analysis of sun-dried *Azolla pinnata* (Bangkok strain) grown in soil-water cultures in the Philippines.

Analyses	Content	Procedure
Moisture	9.62%	
Crude protein	18.16%	Kjeldahl N x 6.25
Crude lipid	1.31%	Ether extract
Ash	21.68%	Residue after ignition at 450°C for 12 hours
Total carbohydrate	58.85%	By difference
Total (gross) energy	3.51 kcal/g	By calculation
Acid-insoluble ash	4.8%	Residue after ashing followed by digestion with 10% HCL
Total carbohydrate	60.1%	Phenol/sulfuric acid method
Available carbohydrate	6.4%	As glucose after amyloglucosidase digestion
True protein	17.6%	Lowry method
Calcium	1.64%	Gravimetrically
Phosphorus	0.56%	Ammonium molybdate method

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*ICLARM Contribution No. 349.

Table 3. Essential amino acid (EAA) profile of *Azolla pinnata* (Bangkok strain)* and quantitative requirements of *Oreochromis mossambicus* (from Jauncey et al. 1983).

Amino acid	EAA content of *A. pinnata* % of sample	 % of crude protein	*O. mossambicus* EAA requirements % of dietary protein
Arginine	1.93	11.14	2.82
Histidine	0.38	2.19	1.05
Isoleucine	0.63	3.64	2.01
Leucine	1.23	7.10	3.40
Lysine	1.00	5.77	3.78
Methionine	0.22	1.27	0.99
Phenylalanine	0.80	4.61	2.50
Threonine	0.49	2.82	2.93
Tryptophan	0.04	0.23	0.43
Valine	0.89	4.62	2.20
Total	7.61	43.39	

*The EAA analysis was performed on a freeze-dried sample which contained 17.32% crude protein (Kjeldahl).

Table 4. Weight losses suffered by *Oreochromis niloticus* fingerlings fed on fresh *Azolla pinnata* (Bangkok strain). Means in any column followed by the same letter are not significantly different and * indicates significant weight loss ($p = 0.05$).

Feeding rate (% body weight/day)	Initial length (cm)	Final length (cm)	Initial weight (g)	Final weight (g)	Total weight loss (g)	% Mortality
10	6.9(0.06)a	6.9(0.18)a	5.80(0.10)a	4.41(0.27)a	−1.39(0.17)*	6.7(3.3)a
20	6.9(0.10)a	6.9(0.05)a	5.72(0.40)a	4.12(0.20)a	−1.60(0.39)*	5.0(2.9)a
Excess	6.9(1.73)a	7.0(0.00)a	5.96(0.31)a	4.50(0.30)a	−1.46(0.10)*	1.7(1.7)a

Table 5. Results of test diets containing-dried *Azolla* fed to *Oreochromis niloticus* fingerlings in duplicate 68-l aquaria (20 fish/aquarium) for 28 days: CP = control feed mix; DAP = dried *Azolla* powder; DA = dried *Azolla;* FCR = feed conversion ratio; G = specific daily growth rate (%); means and standard errors (±).

Treatment no./diet/ feeding rate (% body weight/day)	Initial length (cm)	Final length (cm)	Initial weight (g)	Final weight (g)	G (%)	FCR	Mortality (%)
1. DAP/5%	4.2(0.12)a	4.1(0.11)d	1.38(0.03)a	1.26(0.04)e	−0.34(0.05)d	–	–
2. DAP/excess	4.3(0.06)a	4.3(0.66)d	1.18(0.23)a	1.45(0.04)de	0.83(0.75)cd	117(106)*	–
3. DA pellets/5%	4.1(0.19)a	4.1(0.18)d	1.37(0.09)a	1.23(0.08)e	−0.40(0.01)d	–	–
4. DA pellets/10%	4.4(0.07)a	4.4(0.21)d	1.52(0.12)a	1.36(0.01)e	−0.39(0.26)d	–	–
5. 90% DA: 10% CP/5%	4.0(0.31)a	4.0(0.30)d	1.06(0.01)a	1.29(0.18)e	0.65(0.71)cd	–	–
6. 75% DA: 25% CP/5%	4.1(0.11)a	4.2(0.09)d	1.40(0.09)a	1.40(0.04)de	0.00(0.10)d	–	–
7. 50% DA: 50% CP/5%	4.0(0.05)a	4.7(0.07)cd	1.32(0.11)a	2.04(0.06)cd	0.58(0.41)bc	3.31(0.80)a	2.5
8. 25% DA: 75% CP/5%	4.1(0.27)a	5.2(0.40)bc	1.32(0.28)a	2.32(0.49)bc	2.01(0.00)abc	2.34(0.13)b	–
9. 10% DA: 90% CP/5%	4.0(0.11)a	5.5(0.06)ab	1.34(0.16)a	2.85(0.13)ab	2.71(0.59)ab	1.77(0.27)ab	–
10. Control pellets 5%	4.2(0.13)a	6.0(0.23)a	1.45(0.13)a	3.39(0.36)a	3.03(0.06)a	1.48(0.05)b	–

Means in any column followed by the same letters are not significantly different ($p > 0.05$): *very high due to excess feeding.

Table 6. Results of feeding feed pellet and two *Azolla pinnata* (Bangkok strain) feeds to male *Oreochromis niloticus* in duplicate 225-l aquaria (10/aquarium) for 30 days. G = daily specific growth rate (%); FCR = feed conversion ratio; means and standard errors (±) means in any column followed by the same letter are not significantly different (p >0.05).

Diet and feeding rate	Mean initial length (cm)	Mean final length (cm)	Mean initial weight (g)	Mean final weight (g)	G (g)	FCR	Mortality
Dried *Azolla* pellets fed *ad libitum*	13.9(0.72)a	14.2(0.92)a	45.96(9.48)a	40.51(7.84)a	–0.42(0.04)a	–	0
Fresh *Azolla* fed *ad libitum*	15.4(0.13)a	15.6(0.16)a	61.35(1.75)a	52.98(1.01)a	–0.51(0.03)a	–	5
Control fed at 5% body weight/day	15.0(0.19)a	16.7(0.33)a	58.01(1.80)a	76.12(1.86)b	0.91(0.03)b	5.18(0.20)	0

Importance of Initial Laboratory Feeding for Golden Perch Fry

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Abstract

Golden perch *(Macquaria ambigua)* fry released into rearing ponds at the yolksac resorption stage corresponding to the stage when feeding begins, feed only on small cladocerans (mainly *Moina micrura)* of 350-860 μm although rotifers are often the most abundant zooplankton. Variability of *Moina* density is great and probably accounts for the marked variability in survival rate of fish fry. It is proposed that after the yolksac resorption stage, the fry be fed in the laboratory/hatchery for two to three days on an appropriate diet before release into the nursery rearing ponds.

Diet trials showed that the fry survive and grow better on an artemia nauplii diet than on a size-selected *Moina*-supplemented diet. After laboratory feeding for three days, the fry are more robust and feed on copepods in addition to small cladocerans. This approach may decrease variability in and increase survival rate of the fry.

Introduction

Fry rearing in ponds can be difficult because of the problems of the "critical period" and "point of no return" inherent for some fish fry (Fabre-Domergue and Bietrix 1897; Hjort 1914). These problems appear to be inherent in the golden perch *(Macquaria ambigua)*, an Australian native freshwater fish. Roland (unpublished data) found that the survival rate of golden perch fry was proportional to food density. Arumugam (1986) found that the fry at initial feeding were weak and slow and fed mainly on small cladocerans of about 350-860 μm (mainly *Moina micrura* and first instar *Daphnia carinata)*, although rotifers were often abundant when the fry were released into the ponds (Arumugam and Geddes 1986). *Moina*, the main food item of the fry, is common at this period but its density is highly variable because of the successional pattern of zooplankton in the pond. This variability probably accounts for the marked variability in survival rate of fry, from 1 to 80 % with an average of about 48% (Roland, pers. comm.).

This paper proposes an initial feeding technique so that fry will pass the period of high fry mortality. The technique involves feeding the fry stage when initial feeding commences with a suitable feed for two to three days in the laboratory/hatchery before release into the nursery ponds: (a) to increase survival rate of fry and hence increase production; (b) to lower the variability in survival rate of fry, ensuring a more consistent supply; and (c) to ensure that the fry are better prepared for release into ponds. This technique or its modification has been used successfully for larval/fry rearing (Alikunhi 1957).

The golden perch spawn are kept in hatchery tanks up to the yolksac resorption stage when initial feeding commences (five to six days) and are then released into the pond (Roland 1983). The fry at this stage eat very little food, about 7-21 artemia nauplii per day (Arumugam 1986), making the technique economically feasible. The exorbitant cost of artemia may make the initial feeding technique uneconomical.

The choice of starter feed is of prime importance. Artemia nauplii were used because of their extensive usage for feeding fry (Cheah et al. 1985). An artificial trout feed was also tested because it was relatively cheap and easily available. These two starter feeds were compared with pond zooplankton supplemented with cultured *Moina micrura* and against a control (no food added). Fitness of the fry when released into the pond is important and was assessed by comparing unfed and fed fry using prey preferences and daily food consumption as criteria.

Materials and Methods

The golden perch fry used were about 4.6 mm standard length and were visual predators that generally attacked moving objects. The experiments were carried out at the Inland Fisheries Research Station, Narrandera, NSW, Australia (NIFRS).

Three starter feeds were used: trout feed, artemia nauplii and zooplankton and as a control, no food was added. Trout feed pellets were ground, sieved and those 200-500 μm in size were used. Freshly hatched artemia nauplii (about 400 μm) were prepared from commercial artemia cysts. Zooplankton were collected from ponds at

NIFRS, sieved to obtain a size of 200-500 µm and supplemented with laboratory cultured *M. micrura*.

For each starter feed and the control, five replicates of ten fry each were randomly assigned in separate beakers. Each beaker contained 300 ml of filtered river water that was constantly being aerated. The water in each beaker was changed daily and the fry were fed twice daily. The experiment was run for five days at 20-22°C under 8-hour light and 16-hour dark conditions (fluorescent lighting).

The survival rate of fry in each replicate was noted daily and the means plus standard errors for each starter feed were determined. At the end of the experiment the standard length (mm) and dry weight (µg dried at 60°C for 24 hours) of the surviving fry were determined and their respective means for each starter feed were calculated. A one-way analysis of variance was used to test for differences between starter feeds. For the variates which showed significant differences between starter feeds (length and weight only), Duncan's multiple range test was used to determine the differences between starter feeds (Steele and Torrie 1980).

The common microcrustaceans in the fry rearing ponds, *M. micrura* (350-500µm), *D. carinata* (780-860µm) and calanoids (430-960µm) were used to determine the species preferences of the unfed and fed fry. The prey lengths used were within the size range that the fry could engulf (Arumugam 1986). Thirty individuals of each prey species were placed in a beaker containing a fry individual. The number of individuals remaining for each species after 24 hours was recorded. Ten such feeding trials were carried out simultaneously in an experimental chamber (Arumugam 1986) at 20-22°C for both the unfed and fed fry. Species preference of the fry was determined using the prey preference index for limited density (Chesson 1983).

Five unfed fry were placed in separate beakers in an experimental chamber (Arumugam 1986) at 20-22°C. Forty freshly hatched artemia nauplii were placed in each beaker and the number eaten after 24 hours was recorded. The same procedure was carried out for the fed fry. The daily food consumption was expressed as µg dry weight consumed per day.

Results

The survival rate of the fry using different starter feeds (Fig. 1) showed that fry fed with artemia nauplii had the highest survival rate. A marked decrease in survival rate occurred on day 2 and day 3 for zooplankton and trout feed, respectively. Survival rate remained high after the earlier marked decrease in survival for both zooplankton and trout feed. Statistical analysis showed that the mean length of fry fed with artemia nauplii was significantly longer than the fry fed with the other two starter feeds and the control (Table 1). The mean weights of fry were similar for fry fed with artemia nauplii and zooplankton starter feeds but significantly heavier than the fry fed with trout feed and the control.

The comparisons of prey preferences showed that the fed fry had a higher preference for copepods than the unfed fry (Table 2). The unfed fry had a daily consumption of 33.7µg/day; the fed fry, 53.0µg/day.

Discussion

Artemia nauplii were suitable as a starter feed. The fry of the golden perch had good survival and grew well during the four-day period. Fry fed on zooplankton had good growth but a lower survival rate than those fed with artemia which was probably due to initial feeding problems. Cheah et al. (1985) found a significantly lower survival rate on *Moina* diet as compared to artemia nauplii diet for *Helostoma temmincki*. The poor growth of fry fed on trout feed indicated that the feed formulation as a whole was nutritionally inadequate as a starter feed for golden perch.

The use of the trout feed for the unfed fry emphasizes the importance of the initial feeding period in the laboratory to improve fitness of the fry. The fed fry had a wider spectrum of prey species to capture; they were capable of feeding on the faster moving copepods and also had a higher daily food consumption than the unfed fry. This suggests that after an initial feeding period in the laboratory the fry improved their capture efficiency (Braum 1978; Arumugam 1986). As such the fed fry had better probability of survival when released into the ponds than the unfed fry. It is important that the timing of release into the pond of fry at first feeding stage coincides with abundance and availability of the appropriate size range of prey species. Also, weak fry are prone to predation by insect predators.

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Table 1. Comparisons of mean lengths and mean weights of fry for the three starter feeds and the control using the Duncan's multiple range test. (N.B. a line joining 2 or more values indicate that these values are not significantly different; * = 5% probability).

	Starter feeds			
	Control	Trout feed	Zooplankton	Artemia nauplii
Length (mm)	4.77	4.79	4.76	4.92*
Weight	161.5	161.8	173.0	175.5*

Table 2. Comparisons of prey preference index values (mean ± standard error) of fry at first feed (unfed fry) and fry fed on a trout feed diet for three days (fed fry) for *Moina*, *Daphnia* and calanoids.

	Prey preference index	
	Unfed fry	Fed fry
Moina	0.56 ± 0.10	0.27 ± 0.07
Daphnia	0.43 ± 0.10	0.54 ± 0.08
Calanoid	0.01 ± 0.01	0.19 ± 0.06

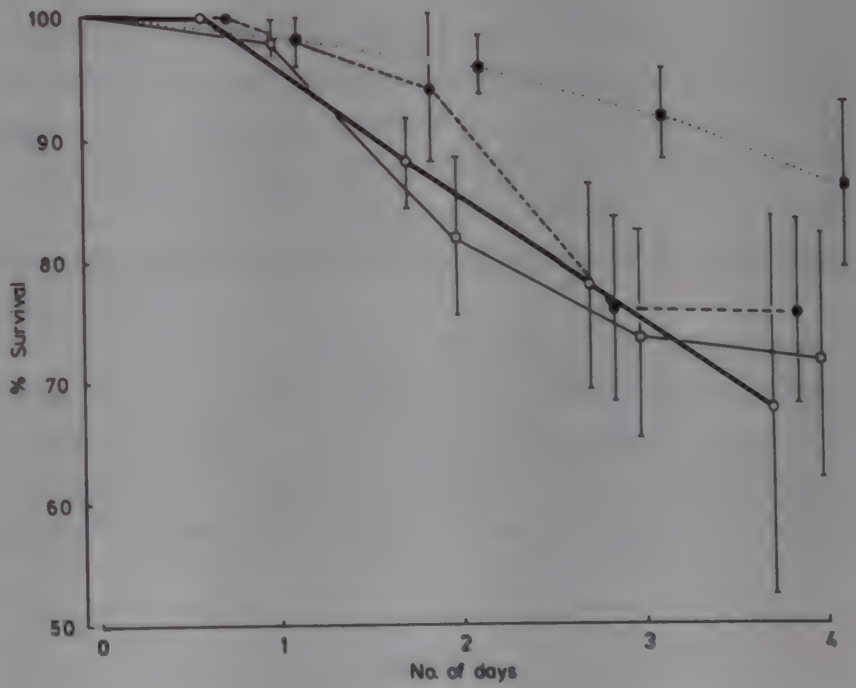

Fig. 1. Percentage survival ($\overline{X}$ ± S.E.; n = 5) of fry for the three starter feeds and the control.

Growth and Survival of Nile Tilapia Fingerlings in Net Cages Without Supplemental Feed in Laguna Lake, Philippines

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Abstract

Oreochromis niloticus fingerlings were stocked at densities of 50, 100, 150 and 200 fish/m^2 in fixed net cages in Laguna Lake, Philippines. These were reared without supplemental feed on 1 April-30 July 1980, 5 August-3 December 1980 and 19 December 1980-19 April 1981. Water temperature, dissolved oxygen, chloride level and pH for the three periods were measured outside the net cages. Growth rates were highest at 50 and 100 fish/m^2 reared between April and July when phytoplankton biomass was high with a peak production observed in June (65.5 g/m^2), and zooplankton biomass ranged from 9.3 to 32.68 g/m^2. Under these conditions, fish initially weighing 1.6-1.7 g grew to a marketable size of 106-124 g in four months. Those stocked at 150 and 200 fish/m^2 had lower weights but still weighed over 80 g. None of those in the other two rearing periods exceeded 50 g after four months. Survival and fish yield during April-July were also higher than for those reared during the other periods.

Introduction

The tilapias are fast becoming important culture species in developing countries. In the Philippines, *Oreochromis niloticus*, introduced from Thailand in 1972 (Balarin and Hatton 1979), is now one of the favorite food fishes and is cultured extensively in lakes and ponds.

The cage culture of *O. niloticus* in Laguna Lake is a relatively new development in the Philippines but it has prospered into a major industry. Delmendo and Baguilat (1974) were the first to report on the cage culture of *O. niloticus* in the Philippines. In 1981, about 1,000 ha of the 90,000-ha lake were devoted to tilapia culture in small pens and cages (Pullin 1981). Nile tilapia is easy to breed and economical to grow in this lake. There are reports, however, of slow growth in some net cage farms during certain months of the year. Growth data are apparently lacking in spite of the proliferation of tilapia cages in Laguna Lake.

This study compares the growth, survival and yield of *O. niloticus* fingerlings in fixed net cages at various stocking densities and different periods of the year.

Materials and Methods

The tilapia fingerlings came from the lake-based nursery of the Binangonan Research Station, Aquaculture Department, Southeast Asian Fisheries Development Center. The parental stock was a cross between a Philippine selection that originally came from Thailand and a strain from Singapore (Bautista, pers. comm.).

The net cages were suspended in fixed bamboo poles and spaced 1 m apart at the west side of Tapao Point in Laguna Lake. Each cage was 1 x 1 x 1.5 m and made of polyethylene netting with 5.4 mm mesh size. The submerged portion of the net cage was 1 m^3.

Water quality parameters (temperature, chloride, dissolved oxygen content, pH) and phytoplankton and zooplankton biomass were measured weekly using standard procedures.

All the three experiments were carried out in the same site and culture net cages. Details of the experimental design are shown in Table 1. There were four stocking density treatments with four replicates each in a completely randomized design. The total length and weight of ten randomly sampled fish were measured at 30-day intervals over a period of 120 days. No artificial feed was given during the experiments. At the end of each experiment, bulk weights and total counts of fish per cage were recorded.

All data on growth, the arc sin square root transformation of survival rates were subjected to one-way analysis of variance. Duncan's new multiple range test was employed to compare the significance of the means of the growth parameters among the stocking densities tested.

Results

In experiment 1 (1 April-30 July 1980), the mean fish weight at stocking density of 50 fish/m^2 was significantly higher than those at 100, 150 and 200 fish/m^2 ($P < 0.05$, Table 2) after 120 days. Mean fish weight at

100 fish/m^2 was also significantly higher than those at 150 and 200 fish/m^2. The difference between those stocked at 150 and 200 fish/m^2 were statistically insignificant ($P > 0.05$). The average growth rate of fish stocked at 50/m^2 was 1.03 g/day, while growth at 100 and 200 fish/m^2 ranged from 0.70 to 0.87 g/day. High survival rates (91-98%) were obtained all stocking densities and were not significantly different ($P > 0.05$, Table 3). Total yield of harvested fish increased with the increase of the stocking density. Highest yield was at 200 fish/m^2 (15.6 kg/m^2) and lowest at 50 fish/m^2 (5.2 kg/m^2). The difference between total yield at 100 and 150a fish/m^2 was insignificant.

In experiment 2 (5 August-3 December 1980), stocking density at 50 fish/m^2 showed significantly faster growth than higher densities of 150 and 200 fish/m^2 ($P < 0.05$, Table 2). No significant difference was found between 50 and 100 fish/m^2 and among 100, 150 and 200 fish/m^2. Mean survival rates were 74-84% and had no significant difference ($P > 0.05$). Like experiment 1, total yield was highest at 200 fish/m^2 (3.9 kg/m^2) and lowest at 50 fish/m^2 (1.6 kg/m^2). No significant differences were found among 50, 100, 150 and among 100, 150 and 200 fish/m^2.

In experiment 3 (19 December 1980-19 April 1981), mean fish weights at 50 and 100 fish/m^2 were significantly higher than those at 150 and 200 fish/m^2 ($P < 0.05$, Table 2) after 120 days. Differences between those at 50 and 100 fish/m^2 and between those at 150 and 200 fish/m^2 were insignificant. Mean survival rates were 68-93% and showed no significant differences. Compared to Experiments 1 and 2, total yields were very low (0.47-0.78 kg/m^2) and had no significant differences.

The physical and chemical parameters of the water measured outside the cages from 1 April 1980 to 15 April 1981 are shown in Table 4.

Discussion

Marked differences in growth and survival of *O. niloticus* fingerlings grown in cages in Laguna Lake were noted in the three experiments conducted during different parts of the year (Table 2, Fig. 1). Fish reared in April-July showed better growth than those reared in August-December or December-April. Fish stocked at the lowest density showed significantly faster growth within four months than at higher densities. Although smaller, fish stocked at higher densities of 100, 150 and 200 fish fish/m^2 were of marketable size (> 80 g) in July. The effect of population density on the growth of some fish has been demonstrated by other workers (Coche 1976; Chua and Teng 1979).

The fast growth of fish in April-July may be attributed to the high primary productivity which reached its peak of 8.8 gC/m^2/day in June 1980 and remained high (2.7-6.0 gC/m^2/day) in July (Nielsen et al. 1981). Phytoplankton biomass at the experimental site was comparatively high with a peak production observed in June (average of 65.6 g/m^2, Fig. 1a). Prior to the increase in primary productivity, there was a significant increase in chloride concentration (Table 4), an indication of seawater intrusion from Manila Bay. Chloride level had a maximum of 1.192 g/l in May and remained high (0.356-1.079 g/l) in June. This was followed by improved clarity of water that stimulated an increase in primary production (Nielsen et al. 1981). Highest water temperature was recorded in April-July, varying from 29 to 33°C. The fast growth and very high yield of fish suggest that the available natural food was enough to meet the requirement of fish stocked as high as 200 fish/m^2.

The stunted growth of fish observed in the other two experiments could be accounted for by the relatively low phytoplankton biomass during the period (Fig. 1a). The phytoplankton biomass in August-December 1980 (Experiment 2) and in December 1980-April 1981 (Experiment 3) were 4.7-11.4 and 5.1-8.9 g/m^2, respectively. Primary productivities of water in August-December 1980 and in December 1980-April 1981 were 0.2-5.9 and 0.5-1.5 gC/m^2/day, respectively (Nielsen, pers. comm.). These were relatively low compared to the observation in April-July 1980. Perhaps the amount of natural food during Experiments 2 and 3 was not sufficient to sustain optimum growth even at a low stocking density of 50 fish/m^2. As observed by Hepher (1972), when the natural food is sufficient only for maintenance, growth ceases entirely. According to Almazan and Boyd (1978), when gross primary production is below 0.48 gC/m^2/day, growth of tilapia stops. Aquino and Nielsen (1983) have likewise shown that the primary production rate of 0.5 gC/m^2/day is the lower limit for tilapia growth in Sampaloc Lake. However, fish growth was better in August-December than in December-April, probably because of the higher zooplankton biomass during August-December (Fig. 1b) and the low water temperature in December 1980-April 1981 (Table 5).

Mouthbrooding females (about 19 g) were observed in some cages in Experiments 2 and 3 during the third month of culture. Precocious breeding of tilapia in natural waters has been associated with inadequate food (Lowe-McConnell 1982).

This study showed that the growth of tilapia in the cages of Laguna de Bay is greatly influenced by seasonality of natural food and temperature. For proper management, the level of production of natural food items, mainly plankton, has to be known. This figure determines the fish yield which may be harvested without affecting the system in a negative way. Artificial food may be required during about eight months of the year when the concentration of plankton is below the level for maximum growth of tilapia in Laguna de Bay.

A continuous study of environmenal conditions in Laguna Lake is necessary now that a barrier between the Lake and the Pasig River, into which it drains (the Hydraulic Control Structure) is operational. Changing patterns of productivity should be monitored to determine the rearing period for *O. niloticus* under the new conditions.

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Table 1. Experimental design, initial stocking density, initial biomass and size of fish stocked.

	Initial stocking density (fish/m^2)	Initial biomass stocked (kg/m^2)	Size of fish stocked			
			Mean weight (g)	S.D.*	Mean length (mm)	S.D.*
Experiment 1 (1 Apr-30 July 1980)	50	0.08	1.69	0.74	47.40	7.40
	100	0.16	1.55	0.61	48.20	8.29
	150	0.26	1.71	0.68	47.82	6.62
	200	0.28	1.40	0.68	45.42	6.55
Experiment 2 (5 Aug-3 Dec 1980)	50	0.07	1.36	0.56	42.73	5.32
	100	0.12	1.25	0.42	41.85	4.22
	150	0.18	1.22	0.41	42.08	4.56
	200	0.27	1.37	0.43	42.82	4.79
Experiment 3 (19 Dec 1980-19 Apr 1981)	50	0.04	0.90	0.44	37.88	6.44
	100	0.09	0.88	0.44	38.95	6.14
	150	0.10	0.68	0.20	35.02	3.57
	200	0.13	0.63	0.20	33.90	4.16

* ± Standard deviation.

Table 2. Experiments 1-3: mean weights (g) of sampled *O. niloticus* cultured at three different periods of the year. Means followed by the same letter(s) are not significantly different ($P > 0.05$).

	Stocking density (fish/m^2)	Days 0	30	60	90	120
Experiment 1 (April-30 July 1980)	50	1.69^{a}	8.50^{a}	20.61^{a}	68.52^{a}	124.83^{a}
	100	1.55^{a}	6.87^{b}	17.17^{b}	67.66^{a}	106.02^{b}
	150	1.55^{a}	6.37^{b}	15.88^{bc}	62.14^{a}	85.53^{c}
	200	1.40^{a}	6.30^{b}	14.97^{c}	49.65^{b}	84.31^{c}
Experiment 2 (5 August-3 December 1980)	50	1.36^{a}	9.65^{a}	17.74^{a}	28.90^{a}	32.40^{a}
	100	1.25^{a}	7.70^{b}	14.91^{b}	25.33^{b}	31.46^{ab}
	150	1.22^{a}	8.22^{b}	14.36^{b}	21.03^{c}	29.12^{b}
	200	1.37^{a}	7.30^{b}	11.73^{c}	18.88^{c}	28.58^{b}
Experiment 3 (19 December 1980-19 April 1981)	50	0.90^{a}	2.98^{a}	4.90^{a}	7.26^{a}	9.66^{a}
	100	0.88^{a}	2.99^{a}	4.89^{a}	6.73^{a}	8.74^{a}
	150	0.68^{b}	2.52^{b}	3.54^{b}	5.06^{b}	6.72^{b}
	200	0.63^{b}	2.15^{b}	3.23^{b}	4.79^{b}	6.34^{b}

Table 3. Mean initial weights, final weights, mean daily growth rate, survival rate and total yield of *O. niloticus* fingerlings cultured at three different periods of the year. Means followed by the same letter(s) are not significantly different ($P > 0.05$).

	Stocking (no. of fish/m^2)	Mean weights Initial (g)	Final (g)	Mean daily growth (g/fish/day)	Mean daily growth rate (%)	Survival (%)	Total yield (kg/m^2)
Experiment 1 (April-30 July 1980)	50	1.69^{a}	124.83^{a}	1.03	61.14^{a}	98.5^{a}	5.21^{c}
	100	1.55^{a}	106.02^{b}	0.87	53.13^{a}	95.5^{a}	9.52^{b}
	150	1.71^{a}	85.53^{c}	0.70	49.36^{ab}	91.1^{a}	11.70^{b}
	200	1.40^{a}	84.31^{c}	0.70	41.33^{b}	97.9^{a}	15.64^{a}
Experiment 2 (5 August-3 December 1980)	50	1.36^{a}	32.40^{a}	0.26	19.56^{a}	83.5^{a}	1.57^{b}
	100	1.25^{a}	31.46^{ab}	0.26	20.37^{a}	84.0^{a}	2.83^{ab}
	150	1.22^{a}	29.12^{b}	0.23	19.20^{a}	74.5^{a}	3.04^{ab}
	200	1.37^{a}	28.58^{b}	0.23	16.54^{a}	77.8^{a}	3.92^{a}
Experiment 3 (19 December 1980-19 April 1981)	50	0.90^{a}	9.66^{a}	0.07	8.06^{a}	80.0^{a}	0.47^{a}
	100	0.88^{a}	8.74^{a}	0.07	7.43^{a}	88.8^{a}	0.68^{a}
	150	0.68^{b}	6.72^{b}	0.05	7.60^{a}	92.5^{a}	0.78^{a}
	200	0.63^{b}	6.34^{b}	0.06	7.64^{a}	68.4^{a}	0.76^{a}

Table 4. Changes in the water temperature, chloride level, dissolved oxygen content and pH outside the net cages. Each value is the monthly measurement taken weekly over the period of the experiments (1 April 1980-15 April 1981). Values in parentheses indicate the ranges.

Months	Water temperature (°C)	Dissolved oxygen content (mg/l)	pH	Chloride (g/l)
April	29.7[b] (29.0-30.0)	6.8[b] (6.1-7.2)	7.8[b] (7.3-8.0)	0.20[b] (0.201-0.210)
May	32.4[b] (31.5-33)	9.1[b] (4.6-11.8)	8.4[b] (7.4-8.9)	0.629[b] (0.200-1.192)
June	30.3[b] (29.0-31.0)	6.4[b] (1.7-10.2)	9.0[b] (8.8-9.2)	0.675 (0.356-1.079)
July	32.0[a] –	3.0[a] –	8.2[a] –	0.573[a] –
August	31.0[c] (30.0-32.0)	5.6[c] (3.8-9.7)	7.8[c] (7.3-8.4)	0.340[c] (0.309-0.395)
September	29.1[c] (27.0-32.0)	4.7[c] (2.6-6.2)	7.55[c] (7.3-7.9)	0.269[c] (0.228-0.297)
October	29.9[c] (28.0-31.0)	4.7[c] (3.9-5.6)	7.97[c] (7.4-8.7)	2.287[c] (0.273-0.297)
November	27.5[c] (26.5-28.5)	5.1[c] (3.1-6.7)	7.1[c] (6.9-7.5)	0.213[c] (0.273-0.297)
December	27.0[b] (26.5-27.5)	5.6[b] (4.9-6.3)	7.2[b] (7.1-7.5)	0.192[c] (0.185-0.196)
January	23.3[c] (22.5-24)	6.8[c] (6.4-7.2)	7.3[b] (7.1-7.5)	0.192[c] (0.181-0.194)
February	25.0[c] (24.0-26.8)	5.4[c] (5.0-6.3)	8.1[c] (7.6-8.6)	0.187[c] (0.181-0.194)
March	25.8[d] (24.0-27.0)	5.5[d] (5.0-6.1)	7.9[d] (7.7-8.4)	0.186[d] (0.185-0.188)
April	28.0[a] –	6.1[a] –	7.9[a] –	0.188[a] –

[a] One measurement.

[b] Mean of 3 measurements.

[c] Mean of 4 measurements.

[d] Mean of 5 measurements.

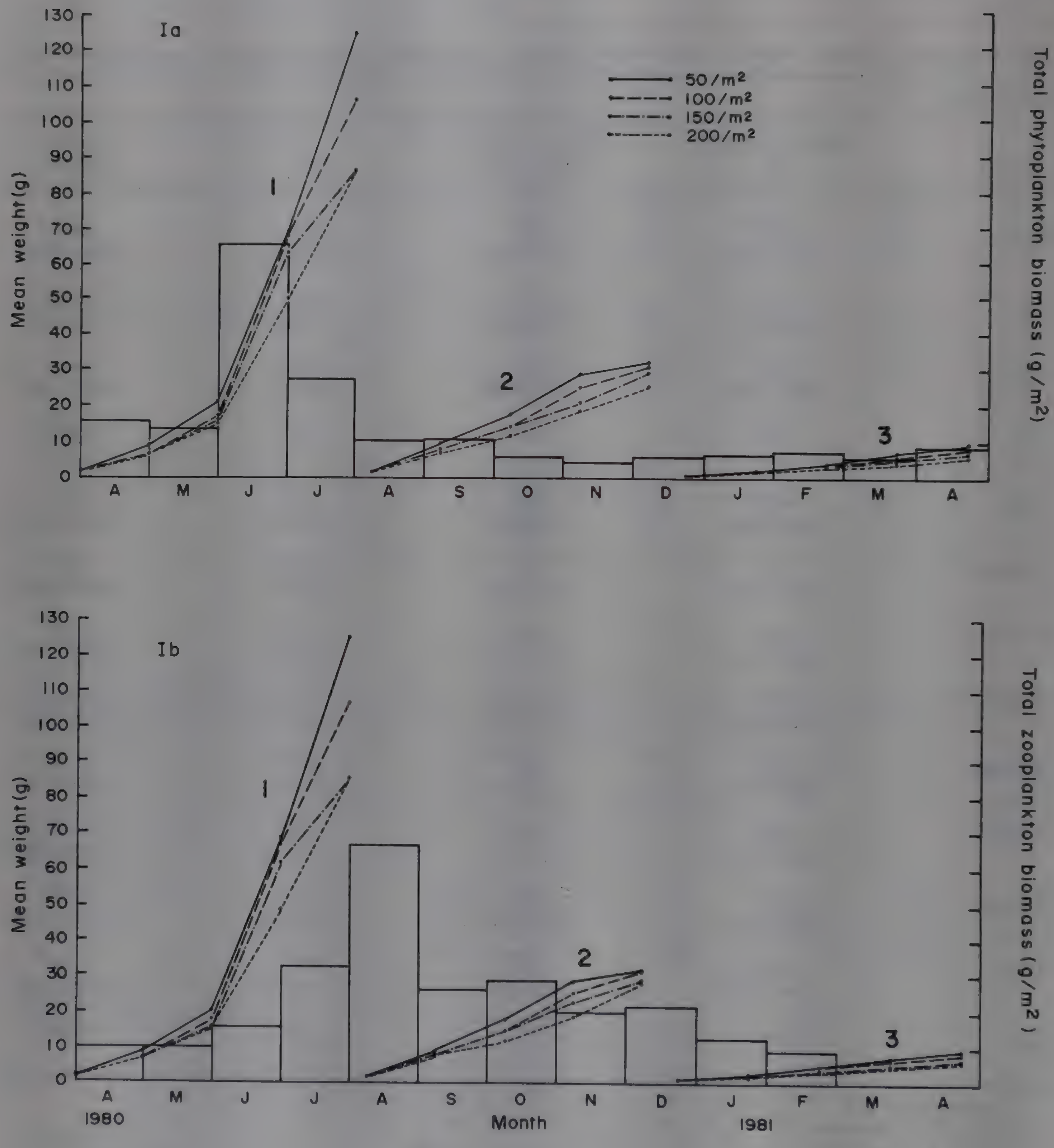

Fig. 1. Phytoplankton and zooplankton biomass in relation to tilapia growth in net cages in Laguna Lake, Philippines.

Formulation and Evaluation of Pelleted Fish Feed Based on Soyabean Meal and Squilla Meal

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Abstract

Utilization of locally available material suitable for feeding carps is one of the easy means to reduce the cost of production. With this objective, two new fish feeds were formulated: deoiled soya flour (pellet SB) and squilla meal (pellet SM). These diets were evaluated in relation to a fishmeal-based diet (pellet FM) by the growth performance of rohu, common carp and silver carp. Each treatment was tried in triplicate in cement cisterns of 25-m^2 area each over 133 days. The fishes were stocked at a total stocking density of 7,200 fingerlings/ha and fed once a day at 5% body weight. The average growth of all the three species grown on fishmeal-based diet was marginally superior to that obtained with the other two diets, without any statistical difference at 5% level of significance. The relative conversion rate was better with pellet FM (1.23) than with pellet SB (1.37) and pellet SM (1.31). The absolute conversion rate (ACR) and specific growth rate (SGR) studies confirmed the results of field observations in all the three diets in the case of silver carp, while in rohu pellet SB yielded slightly better ACR and SGR than the other two diets. In the case of common carp, the ACR and SGR were highest with pellet FM feed. Although the net return was highest with pellet FM due to better survival and growth, the nearly similar relative and absolute conversion rates of the three diets indicate that the two new sources of protein evaluated could be used for partial replacement of fishmeal rather than its complete replacement.

Introduction

Use of locally available material for feeding carps is an easy way to reduce feed production costs. This report attempted to evaluate comparatively one animal protein source (squilla meal) and one plant protein source (soyabean meal) with fishmeal. The squilla meal was selected for its low cost, abundance and local availability, while the soyabean meal was selected for its high protein content and increasing availability. The performances of squilla meal-based diet (pellet SM) and soyabean meal-based diet (pellet SB) were compared with the fishmeal-based diet (pellet FM) developed earlier at the College, through feeding trials on silver carp, rohu and common carp.

Materials and Methods

The feed ingredients were procured from the local market. Their proximate composition was determined by the methods of AOAC (1975) to estimate moisture, crude protein and crude fat, Pearson (1976) for crude fiber and Hastings (1976) for nitrogen-free extract. The quantity of ingredients in each type of pellet was regulated in such a way as to obtain a protein level of about 31%. The percentage of the ingredients in the three feeds is indicated in Table 1. The required quantities of the ingredients were mixed with water at a ratio of 1:0.8 and kneaded well to obtain a consistent dough which was autoclaved at 105°C for 30 min. The cooled dough was passed through a pelletizer to obtain pellets of 3-mm diameter, then sundried and stored in heavy duty plastic bags.

The experiment was conducted for 133 days in nine cement cisterns, each 25 m^2 (5 x 5 x 1 m) and provided with a soil bed of 15 cm. Before the experiment, each cistern was dried, the bottom soil raked and limed at 500 kg/ha. Five days after liming, all the cisterns were filled with water and uniformly fertilized with poultry manure at 2,000 kg/ha a week prior to stocking. Each cistern was stocked with fingerlings of silver carp [*Hypophthalmichthys molitrix* (Val.)], rohu *(Labeo rohita)* and common carp [*Cyprinus carpio* (Linn.)] at the ratio 1:1.2:1.4 and total stocking density of 7,200 fingerlings/ha. The fishes were fed daily at 5% body weight which was determined by sampling every fortnight.

To determine the absolute conversion rate, a short-term experiment of 42 days was conducted in nine cement tubs, each measuring 60 x 60 x 90 cm, without soil base. Three tubs were utilized for each species of fish, with one tub having only one specimen of that species and receiving only one type of feed. The fishes were fed at 5% body weight. Water in the tubs was renewed every other day.

The stability of feeds was determined by the wet durability test of Hastings (1964) over a period of seven hours.

Water samples were collected fortnightly from the cisterns and analyzed for pH, dissolved oxygen, dissolved carbon dioxide, dissolved organic matter, total alkalinity, phosphate-phosphorus, nitrate-nitrogen and ammonia-nitrogen by APHA (1975) methods.

The two-way analysis of variance technique (Snedecor and Cochran 1968) was employed to find the significant difference, if any, in the final average growth attained by the three species of carps fed on different feeds at 5% level of significance.

Results and Discussion

The proximate compositions of the ingredients and the formulated feeds are presented in Tables 2 and 3, respectively. These findings proximate those of earlier workers (Jayaram and Shetty 1981; Mathew et al. 1982; Viola and Arieli 1983). In terms of caloric content, SB had the highest Kcal/g while SM had the lowest. The low carbohydrate and high fiber content of squilla meal could be responsible for the slightly lower caloric value of SM.

In the diets of catfish, a fiber content of 21% reduced the nutrient intake and impaired digestibility, while a lower fiber content of less than 8% resulted in good growth (National Research Council 1977). However, in this study, SM with a fiber content of 16.62% yielded better growth than SB with rohu under field conditions. Similar observations were made by Anil (1981) and Nandeesha et al. (unpublished data) with rohu only.

The three diets differed in stability in seven hours. After one hour, SM and FM were found to have slightly higher stability (91.55% and 91.46%, respectively) than SB (88.42%). The stability of all three declined progressively with time. Because of the slow feeding habit of carps, feeds stable for at least one hour, without much disintegration, are required. All the three newly-developed feeds possess this quality.

Silver carp showed initially a more positive response to SB than to the other two diets (Fig. 1). However, after the 98th day, its growth with FM proved to be decidedly better. The response of silver carp to SM remained poor throughout. The average daily increment in growth for the entire period was highest with FM 1.99 g/day, while it was 1.85 g/day and 0.91 g/day with SB and SM, respectively. The short-term experiment ascertained similar growth increment and absolute conversion rate of the feeds (Table 4).

In contrast, rohu showed comparatively good growth with SM till the 98th day, after which it grew better with FM but poor with SB (Fig. 2). The overall daily increase in weight of rohu was 1.08 g with FM, 1.04 g with SM and 1.01 g with SB, without any statistically significant variations. However, the pattern was a little different in the laboratory experiment where SB recorded better conversion rate and specific growth rate. (Table 5).

Like rohu, the common carp did not show any marked difference in growth between the diets. Although FM ultimately proved superior, lead in growth rate alternated between FM and SB (Fig. 3). The daily growth rates were 2.68 g with FM, 2.58 g with SB and 2.51 g with SM. The difference in final average weight between the treatments and average daily growth rates obtained in the short-term experiment to determine the absolute conversion rate were insignificant. However, in terms of specific growth rate, SM and SB proved equal. The absolute conversion rate obtained with SM was found to be slightly better than that of SB (Table 6).

In general, the growth of all the species of carps studied was found better with FM. Fishmeal-based feeds have been known to yield good growth in fishes, fishmeal being rich in all dietary essentials (Andrews and Page 1974). On the other hand, soyabean meal is known to be deficient in lysine and methionine, two of the essential amino acids. Viola et al. (1981) succeeded in achieving good growth of carps by partially replacing fishmeal with soyabean meal and adding 5% oil and methionine. Further studies in Israel (Viola et al. 1982) indicate that to obtain the same growth with fishmeal-based feed, the soyabean meal-based feed has to be supplemented with methionine, lysine and 10% oil. Although the activity of trypsin inhibitors in soyabean meal is also considered a factor in growth depression, Viola and Arieli (1983) opined that in properly heated soyabean-based diets, inadequate lysine not the trypsin inhibitors is the main reason for poor growth. Recent studies (Wilson et al. 1981; Wilson and Poe 1985) suggest that other than the activity of trypsin inhibitors and lysine deficiency, soyabean meal also contains additional antinutritional factors which are yet to be elucidated.

The lower growth rate of fishes obtained in the present study with soyabean meal-based diet could be attributed to lysine and methionine deficiency and unknown antinutritional factors, but not to the activity of trypsin inhibitors, since the food was adequately cooked before pelletization. Smith (1977) indicated that complete destruction of antinutritional factors is possible at 175-195°C, at which temperature, however, amino acid availability may decrease, according to Dabrowski and Kozak (1979). The better absolute conversion rate obtained in rohu with SB indicates its suitability for incorporation in the diet for this herbivore. Rohu is known to grow well with plant protein sources (Anil 1981) but this needs confirmation in the light of growth-depressing factors in soyabean meal.

The lower growth rate of fishes obtained with the squilla meal-based diet appears mainly due to its low energy value and high chitin content. Oke et al. (1977) investigated the nutritional value of shrimp wastes as animal feed and reported a protein efficiency ratio of 2:1

when fed to rats at 10% level. Their investigation indicated that shrimp wastes contained 78.7% digestible protein and were rich in lysine and methionine. Shrimp meal is known to promote growth when present in the feed at 5% level and to inhibit growth at 20% level (Fowler and Banks 1976).

In this experiment, squilla meal constituted 30% of SM and the growth data and absolute conversion rate obtained do not indicate severe inhibition of growth. This could be due to the use of whole squilla (*Squilla* sp.) in the meal as compared to shrimp waste, which consists of only inedible portions of the shrimp. This view is further supported by the findings of Mathew et al. (1982) who obtained a protein efficiency ratio for squilla protein equal to that of casein in rats.

The data on overall survival, cost of feed, relative conversion rate, net fish production and economics of production are presented in Table 7. Survival was highest with FM and lowest with SM. Water analysis revealed that dissolved oxygen (DO) became the limiting factor in all the treatments after the 70th day and its depletion was more pronounced and prolonged in the SM treatment, causing mortality in that treatment. The decline in DO could be due to the high biochemical oxygen demand of accumulated organic matter. Other parameters of water quality were not deterimental to fish life (Table 8). Water temperature varied from 26 to 30°C during the experiment.

The estimated annual net fish production was decidedly higher with FM (Table 7). The relative conversion rate was also best with FM. In respect of cost of feed SB was costliest followed closely by FM while SM the cheapest. The same trend was seen in cost of production per kg. Even though the cost per kg of fish produced was decidedly lowest with SM, in terms of overall economics, FM proved to be clearly superior. It is clear that due to the low cost of production of SM the production economy with it could be improved if better survival can be ensured.

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Table 1. Proportion of ingredients in formulated feeds.

	Percentage of ingredients					
Type of feed	Fishmeal	Soyabean meal	Rice bran	Squilla meal	Tapioca flour	Groundnut oil cake
FM	25	–	40	–	10	25
SB	–	23	42	–	10	25
SM	–	–	30	30	10	30

Table 2. Composition of feed ingredients.

	Percentage composition by weight						
Ingredients	Dry matter	Crude protein	Crude fat	Crude fiber	Nitrogen free extract	Ash	Caloric content (kcal/g)
Fishmeal	91.67	61.12	4.38	0.98	5.09	20.10	3.65
Soyabean meal	97.07	62.41	1.41	0.42	27.36	5.47	4.34
Squilla meal	88.78	48.04	2.63	13.53	6.59	17.99	2.76
Rice bran	89.67	9.20	1.35	31.05	28.23	19.84	1.71
Groundnut oil cake	92.06	54.29	7.69	4.62	19.43	6.02	4.18
Tapioca flour	95.90	7.80	1.30	0.43	73.22	13.15	3.83

Table 3. Proximate composition of feeds.

Pelleted feed	Percentage composition by weight						Caloric content (kcal/g)
	Dry matter	Crude protein	Crude fat	Carbohydrate (NFE)	Crude fiber	Ash	
FM	91.12	31.50	4.96	27.30	11.54	15.82	3.11
SB	90.37	31.82	2.93	31.65	12.69	11.28	3.12
SM	96.18	31.02	2.93	26.62	16.62	18.99	2.88

Table 4. Absolute conversion factor of feed pellets for silver carp.

Pellet	Days after stocking	Feed consumed/ week (g)	Weight of fish (g)	Growth increment/ week (g)	Conversion factor/ week	Conversion factor for 42 days	Average growth day (g)	Specific growth rate (%/day)	Average SGR (%/day)
FM	0	–	32.0	–	–			–	
	7	9.6	36.5	4.5	2.13			1.88	
	14	10.8	41.5	5.0	2.16			1.83	
	21	12.0	47.0	5.5	2.18	2.46	0.77	1.78	1.67
	28	14.4	53.0	6.0	2.40			1.72	
	35	16.2	58.5	5.5	2.95			1.41	
	42	17.4	64.5	6.0	2.90			1.40	
SB	0	–	35.0	–	–			–	
	7	10.8	39.0	4.0	2.70			1.55	
	14	12.0	43.5	4.5	2.67			1.56	
	21	13.2	48.5	5.0	2.64	2.94	0.68	1.58	1.42
	28	14.4	52.5	4.0	3.60			1.13	
	35	15.6	57.5	5.0	3.12			1.30	
	42	17.4	63.5	6.0	2.90			1.42	
SM	0	–	30.0	–	–			–	
	7	9.0	33.0	3.0	3.00			1.36	
	14	10.2	36.5	3.5	2.91			1.44	
	21	10.8	40.0	3.5	3.09	3.02	0.55	1.31	1.36
	28	12.0	44.0	4.0	3.00			1.36	
	35	13.2	48.5	4.5	2.93			1.39	
	42	14.36	53.0	4.5	3.19			1.27	

Table 5. Absolute conversion factor of feed pellets for rohu.

Pellet	Days after stocking	Feed consumed/ week (g)	Weight of fish (g)	Growth increment/ week (g)	Conversion factor/ week	Conversion factor for 42 days	Average growth day (g)	Specific growth rate (%/day)	Average SGR (%/day)
FM	0	–	29.0	–	–			–	
	7	9.0	33.0	4.0	2.25			1.85	
	14	10.2	37.5	4.5	2.27			1.83	
	21	11.4	42.5	5.0	2.28	2.45	0.71	1.79	1.69
	28	12.6	47.5	5.0	2.52			1.59	
	35	14.4	53.5	6.0	2.40			1.70	
	42	16.2	59.0	5.5	2.95			1.40	
SB	0	–	27.0	–	–			–	
	7	8.4	30.5	3.50	2.40			1.74	
	14	9.0	34.5	4.00	2.25			1.76	
	21	10.2	39.5	5.0	2.04	2.36	0.69	1.93	1.74
	28	12.0	45.0	5.5	2.18			1.86	
	35	13.8	50.0	5.0	2.76			1.51	
	42	15.0	56.0	6.0	2.50			1.62	
SM	0	–	26.0	–	–			–	
	7	7.8	29.0	3.0	2.60			1.56	
	14	9.0	31.0	2.0	4.50			0.95	
	21	9.6	34.5	3.5	2.74	3.01	0.50	1.53	1.41
	28	10.2	38.5	4.0	2.55			1.57	
	35	11.4	42.5	4.0	2.85			1.41	
	42	12.6	47.0	4.5	2.80			1.44	

Table 6. Absolute conversion factor of feed pellets for common carp.

Pellet	Days after stocking	Feed consumed/ week (g)	Weight of fish (g)	Growth increment/ week (g)	Conversion factor/ week	Conversion factor for 42 days	Average growth day (g)	Specific growth rate (%/day)	Average SGR (%/day)
FM	0	–	36.0	–	–			–	
	7	10.8	41.0	5.0	2.16			1.86	
	14	12.6	46.5	5.5	2.29			1.83	
	21	14.4	51.5	5.0	2.88	2.47	0.87	1.46	1.67
	28	15.6	57.5	6.0	2.60			1.57	
	35	17.4	64.5	7.0	2.49			1.64	
	42	19.2	72.5	8.0	2.40			1.67	
SB	0	–	37.0	–	–			–	
	7	11.4	41.5	4.5	2.53			1.64	
	14	12.6	45.5	4.0	3.15			1.32	
	21	13.8	50.0	4.5	3.07	2.94	0.71	1.35	1.41
	28	15.0	55.5	5.5	2.73			1.49	
	35	16.8	61.0	5.5	3.05			1.35	
	42	18.6	67.0	6.0	3.10			1.34	
SM	0	–	34.0	–	–			–	
	7	10.2	38.0	4.0	2.56			1.59	
	14	11.4	41.5	3.5	3.26			1.26	
	21	12.6	46.0	4.5	2.80	2.82	0.65	1.47	1.41
	28	13.8	51.0	5.0	2.76			1.47	
	35	15.6	56.0	5.0	3.12			1.34	
	42	16.8	61.5	5.5	3.05			1.34	

Table 7. Fish survival and relative conversion rate and economics of production of feeds.

Feed	Cost/kg	Total feed used (kg)	Net increase in weight (kg)	Relative conversion rate (%)	Total cost (Rs.)	Cost of production/kg fish (Rs.)	Per cent survival	Net fish production (kg/ha/yr)	Cost of feed/ha/yr (Rs.)	Value of fish yield/ha/yr at Rs. 5/kg (Rs.)	Net income/ha/y (Rs.)
FM	2.06	12.94	10.51	1.23	26.66	2.54	68.20	3,847.23	9,757.69	19,236.15	9,478.46
SB	2.23	11.52	8.44	1.37	25.69	3.04	59.00	3,087.43	9,397.49	15,437.15	6,039.66
SM	1.41	7.02	5.34	1.31	9.90	1.85	46.67	1,954.31	3,622.50	9,771.56	6,149.06

Table 8. Range and average value of chemical parameters of water with feed pellets.

Parameter	FM	SB	SM
pH	8.00 – 8.87 (8.46)	8.00 – 8.67 (8.27)	8.00 – 9.00 (8.51)
Oxygen (ppm)	2.93 – 7.47 (4.82)	1.87 – 7.20 (4.56)	1.60 – 9.20 (5.31)
Carbon-dioxide (ppm)	0.00 – 8.67 (4.88)	2.67 – 7.33 (4.71)	0.00 – 9.20 (2.29)
Alkalinity (ppm)	42.67 – 72.67 (58.73)	44.67 – 73.33 (61.40)	39.00 – 81.80 (66.64)
Dissolved organic matter (ppm)	8.03 – 16.67 (12.76)	6.73 – 18.20 (13.09)	3.20 – 17.40 (12.18)
Phosphate-phosphorous (mg/l)	0.05 – 0.30 (0.16)	0.03 – 0.32 (0.15)	0.02 – 0.18 (0.12)
Nitrate-nitrogen (μg at N/l)	2.07 – 13.71 (7.24)	0.88 – 11.72 (5.89)	2.59 – 7.73 (5.08)
Ammonia-nitrogen (μg at N/l)	1.08 – 21.30 (9.31)	1.52 – 26.81 (10.01)	1.14 – 35.07 (11.53)

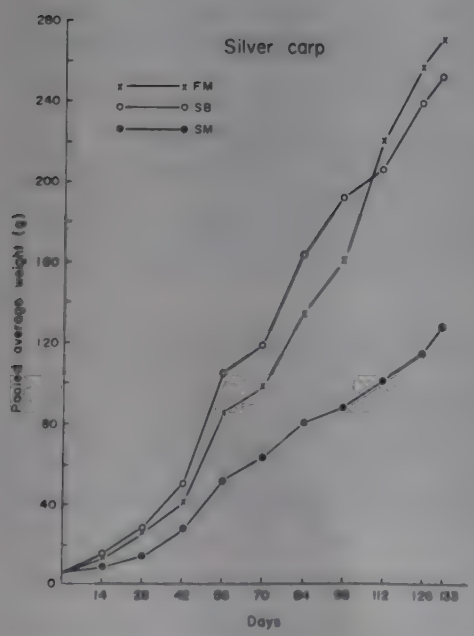

Fig. 1. Average weight of silver carp fed different pelleted feeds.

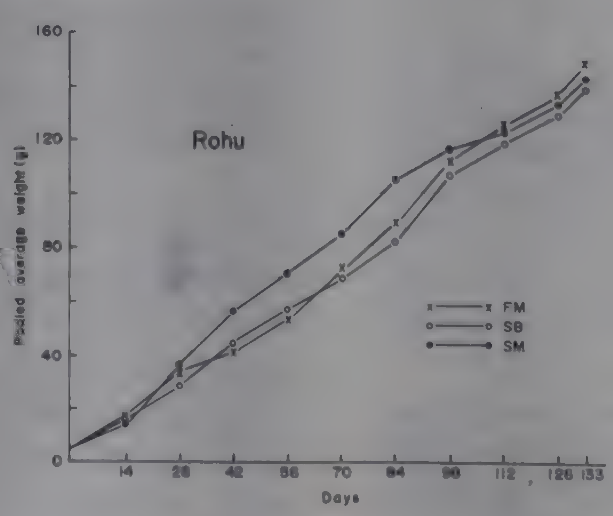

Fig. 2. Average weight of rohu fed different pelleted feeds.

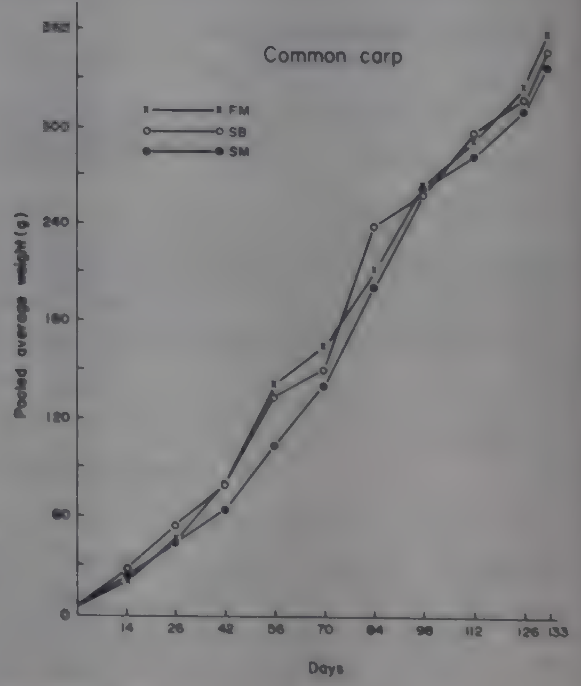

Fig. 3. Average weight of common carp fed different pelleted feeds.

Effect of Amino Acid Supplementation and Vitamin Level on the Growth and Survival of Milkfish *(Chanos chanos)* Fry

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Abstract

The ability to utilize crystalline amino acids varies among different species of fish. To assess this ability in milkfish fry, a study was conducted to determine the response of the fish to amino acid supplementation of a corn-gluten meal-based diet containing 40% protein. Two practical diets, one supplemented with vitamins at moderate levels and the other with high levels of vitamins, were used as controls. Growth and the efficiency of feed conversion were significantly improved ($P < 0.01$) when corn-gluten meal was supplemented with the most limiting amino acid, lysine. Supplementation with five other essential amino acids, deficient relative to the amino acid profile of whole milkfish fry, did not further improve both parameters of measure after 16 weeks of exposure. Growth, survival and efficiency of feed conversion were not affected by the level of vitamins in the diet. No significant difference ($P > 0.05$) in the survival of milkfish fry was observed in any of the treatments but growth and efficiency of feed conversion of fish receiving the corn-gluten meal diets were significantly lower ($P < 0.01$) than those receiving the practical diets. It is concluded that milkfish fry can efficiently utilize crystalline amino acids.

Introduction

Milkfish *(Chanos chanos* Forskal*)* is a major cultured species in the Philippines, Taiwan and Indonesia, where over 300,000 t are produced annually. At present, almost all the fry supply come from the wild. With the increasing needs of growing populations in the region for food, milkfish culture has intensified through the years with corresponding demand for milkfish fry. Major losses of milkfish fry occur in the fry dealer's warehouse and nursery ponds where the fish is most vulnerable to predation and adverse environmental conditions. Thus the development of a technology for rearing milkfish fry in a controlled environment while providing for a nutritionally adequate diet has become a major research thrust.

Many attempts have been made to develop artificial diets for milkfish fry, but until now, improving the survival rates of fry fed artificial diets remain a big challenge (Benitez 1984; Camacho and Bien 1983). Recently, Alava (unpublished data) obtained a survival rate of more than 90% of milkfish fry reared in more than a month. It appears that the major difference between the diets she used and those used by others is the tremendously higher vitamin levels. It is possible that the high survival rate resulted from an allowance provided for vitamin leaching, a serious potential problem because feeds for fry, which weigh only a few mg, are prepared in very fine particles.

Different species of fish vary in their ability to utilize crystalline amino acids. Many researchers have demonstrated the positive growth response of various salmonid species to amino acid supplementation and to amino acid test diets (Rumsey and Ketola 1975; Ogata et al. 1983). Salmonids fed various protein sources supplemented with amino acids have comparable growth as those receiving a fishmeal diet. Carp, on the other hand, is unable to grow on diets with protein component replaced by a mixture of amino acids similar in amino acid profile (Aoe et al. 1970). When the crystalline amino acids were coated with casein, growth was comparable to that of a casein-gelatin diet (Murai et al. 1981). A recent study by Murai and co-workers (1982) suggests that the poor utilization of crystalline amino acids by carp is due to the excretion, rather than catabolism, of these amino acids.

Knowledge of the ability of fish to utilize crystalline amino acids are of primary importance in formulating diets for experiments and for commercial fish production. The ability of fish to utilize crystalline amino acids helps in the determination of the requirement level for essential amino acids and improves the use of various protein sources through amino acid supplementation, thereby reducing reliance on expensive fishmeal. Since fishes differ in their ability to respond to the supplementation of diets with crystalline amino acids (Cowey and Sargent 1979), it is of great importance to determine the effect of amino acid supplementation on the ability of milkfish to utilize a plant protein source.

The effect of supplementing corn-gluten meal with the most limiting amino acid and with a combination of amino acids, to proximate the amino acid profile in milkfish fry itself, was investigated. Furthermore, the effect of megadoses of vitamins on a practical diet as control was examined.

Materials and Methods

Milkfish fry from the wild averaging 7 mg were reared in 59-l rectangular glass tanks. The fry were acclimated for a week during which they were fed a practical diet at 3% of body weight daily. Each tank was supplied with aerated recirculating water at 28°C at the rate of 1.5 l/min. The water, maintained at 18-22 ppt salinity, was sand-filtered and passed through a 75-cm aeration column consisting of 1.28-cm PVC rings compacted in a 10.5-cm PVC tube. Tanks were cleaned everyday by siphoning off food particles and feces and replacing the siphoned water.

Fish were fed to satiation ten times daily during weekdays and eight times daily during weekends, between 8 a.m.and 4 p.m. The amount of feed given was about 10% of body weight. Mortalities during the first three days were replaced. Fifty fish, randomly selected from each tank, were weighed every two weeks.

Five experimental diets (Table 1) were prepared and each fed to four groups of 220 fish. Diet 1 consisted of only corn gluten meal as source of protein; diet 2 was similar to diet 1 except for the supplementation of the most limiting amino acid, lysine; and diet 3 contained a supplement of lysine and five other essential amino acids found to be more than 10% less than the level of these amino acids observed by Coloso et al. (1983) in milkfish fry. Glu and Glu.HCl were added to make the diets isonitrogenous and isochloridic.

A practical diet similar to the one formulated by Lim and Alava (unpublished data) was used as control. This diet was observed to sustain a survival rate of more than 90% of milkfish fry reared in a controlled environment for over a month. Two experimental treatments were based on the practical diet: diet 4 contained relatively high levels of vitamins, similar to the one used by Alava (unpublished data), while diet 5 contained a vitamin mix similar to the one used by Chiu et al. (1984).

Proximate analyses showed that the corn gluten meal diets contained 40.8% protein, 14.9% fat and 6.7% ash, while the practical diets contained 28.5% protein, 6.7% fat and 24.5% ash.

Results

Growth and efficiency of feed conversion were significantly improved ($P < 0.05$) by adding the most limiting amino acid, lysine, to corn-gluten meal in diet 2 (Table 2). Supplementing with other essential amino acids that were more than 10% lower than levels found in milkfish fry (diet 3) did not further improve both parameters of measure ($P > 0.05$). Differences between the growth of fry fed the basal corn-gluten meal (diet 1) and the amino acid-supplemented diet (diet 2 and 3) started to show only after 10 weeks of exposure to these diets (Fig. 1). No pathological symptoms resulting from the lysine-deficient diet (diet 1) was observed after 16 weeks.

No significant difference ($P > 0.05$) in survival rates resulting from the dietary treatments was observed (Table 2). Although percentage survival at the end of 16 weeks ranged from 41 to 50%, it appears that this is not an effect of the dietary treatments. Fig. 2 shows that up to nine weeks of exposure to the diets, survival in all treatments were above 90%. It appears that the drastic drop in the survival rates in all treatments after nine weeks of rearing was due to bacterial infection, evidenced by the arrest of mortalities for several days after a 2.5-ppm antibiotic (oxytetracycline-based) treatment on week 13. Subsequent mortalities occurred after a sampling day, as the weighing of fish may have resulted in stress.

Growth and survival of fish fed diets 4 and 5 (Table 2) did not significantly ($P > 0.05$) improve both parameters of measure. Figs. 1 and 2 show that the growth and survival pattern of fish fed high (diet 4) and moderate (diet 5) levels of vitamins were very similar.

Fish fed corn-gluten meal-based diets (diets 1-3) did not grow as well as those fed the practical diets ($P < 0.05$). Within two weeks, fish fed the practical diets were schooling particularly during feeding. In contrast, no common group behavior was observed in fish fed the corn-gluten meal-based diets. One or two distinctly larger fish were observed in tanks under this treatment. On the other hand, fish fed practical diets had a relatively homogeneous size distribution, probably a consequence of their schooling behavior.

Discussion

It appears that milkfish can utilize crystalline amino acid better than carp. This finding provides a basis for developing test diets for amino acid requirement studies of milkfish and for supplementing protein sources with crystalline amino acids.

Supplementation of corn gluten meal-based diet with the most limiting amino acid, lysine, improved the growth of milkfish fry by more than 70%. Supplementing with other essential amino acids less than the level found in the tissues of milkfish did not further improve growth. In contrast, Atlantic salmon and rainbow trout did not respond to diets supplemented with the first or second limiting amino acids, while a marked improvement in growth was observed when the diets were provided with multiple supplements of amino acids to simulate the amino acid pattern of isolated fish protein or of trout egg (Rumsey and Ketola 1975).

Milkfish fry did not appear to require megadoses of vitamins for maximum growth and survival. The fry were fed ten times a day so that the feed provided was consumed within a minute. Feeding many small meals spread in a day may be more advantageous in improving the utilization of nutrients and minimizing the leaching of vitamins and crystalline amino acids in fry feeds. Yamada et al. (1981) observed better utilization of free amino acids when carp fry were fed diets containing large amounts of free amino acids at 18 feedings over 24 hours compared to three feedings.

Acknowledgements

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Table 1. Composition of experimental diets.

Ingredients	Corn-gluten meal-based (Diets 1-3) g/100 g diet	Control (Diets 4-5) g/100 g diet
Corn-gluten meal	65	10.2
Fish meal		30
Shrimp head meal		16
Soybean meal		10
Rice bran		12
Wheat flour		10.8
Amino acid mix/CMC[a]	12	
Soybean oil	5	
Cod liver oil	5	3
Vitamin mix[b]	4	4
Mineral mix[c]	4	4
Corn starch	5	

[a] Consisted of: L-amino acids in g per 100 g diet: diet 1-none; diet 2-Lys, HC1, 2.8; diet 3-Lys, HC1, 2.8; Arg. HC1, 1.0; His, HC1, H_2O, 0.2; Ile, 0.3; Thr, 0.3; Trp, 0.3; Glu.HC1 and Glu were added to make the diets isonitrogenous and isochloridic. Carboxymethylcellulose was used as a filler.

[b] Supplied in mg/kg: thiamin.HC1, 218 (50); riboflavin, 725 (50); pyridoxine.HC1, 173 (40); vitamin B_{12}, 0.3 (0.2); nicotinic acid, 2901 (200); D-calcium pantothenate, 1016 (200); D-biotin, 21.8 (4); inositol, 14507 (2000); folic acid, 54.4 (15); choline chloride, 29653 (3000); L-ascorbic acid, 7253 (3000); retinyl palmitate B-carotene, 363 (10); cholecalciferol, 36.5 (10); dl-α-tocopherol, 1451 (320); menadione, 173 (20). Levels shown in parenthesis are those used in diet 5.

[c] Supplied in g/kg of diet: $CaHPO_4.2H_2O$, 30; $NaHCO_3$, 1.2; $FeSO_4.7H_2O$, 0.8; $AlK(SO_4)_2.12H_2O$, 0.02; $ZnSO_4.7H_2O$, 0.36; $CuSO_4.5H_2O$, 0.06; $MnSO_4.4H_2O$, 0.30; KI, 0.012; $CoC1_2.6H_2O$, 0.02; Na_2SeO_3, 0.012; KH_2PO_4, 1.2; $MgSO_4.7H_2O$, 2.4.

Table 2. Effect of amino acid supplement and various vitamin levels on the growth, survival and feed conversion in milkfish fry.

Diet No.	Treatment	Weight gain*, ** (mg/fish)	Feed conversion** (g feed/g gain)	Survival (%)
1	Basal corn-gluten meal (CGM) diet	272 ± 36^{a}	1.06 ± 0.06^{a}	44.7 ± 2.1^{a}
2	Basal CGM diet + lysine	468 ± 64^{b}	0.86 ± 0.04^{b}	60.2 ± 2.2^{a}
3	Basal CGM Diet + lysine + 5 amino acids	499 ± 19^{b}	0.84 ± 0.03^{b}	42.6 ± 1.9^{a}
4	Practical diet with high vit. supplement	679 ± 20^{c}	0.68 ± 0.02^{c}	45.0 ± 0.6^{a}
5	Practical diet with moderate vit. supplement	626 ± 26^{c}	0.66 ± 0.05^{c}	41.1 ± 6.3^{a}

*Average initial weight 9.3 mg, 220 fish/aquarium.

**Values represent the mean ± SEM of four tanks of fish. Values with the same letters in the superscript are not significantly different ($P > 0.05$). Data from a sixteen-week growth period.

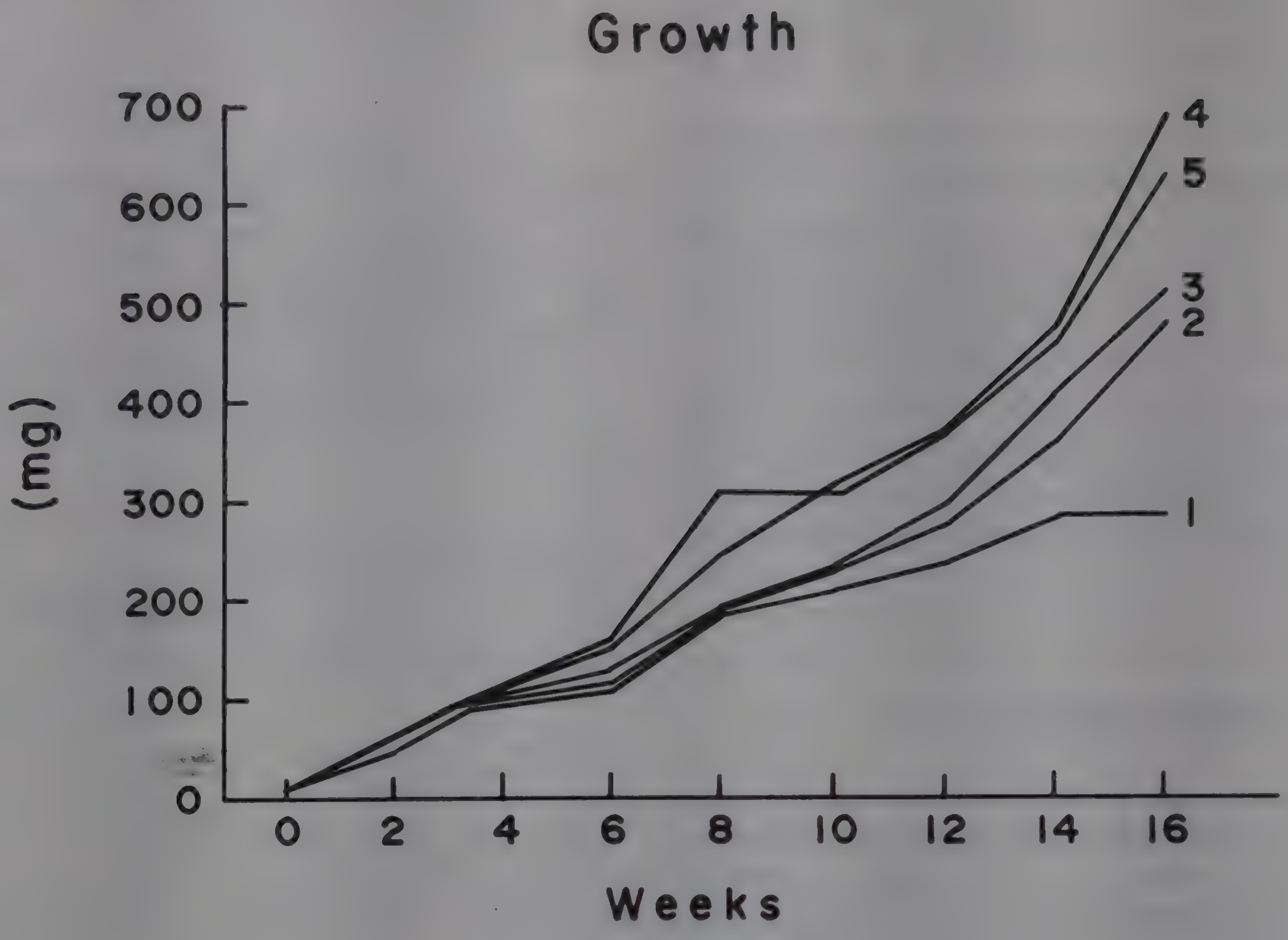

Fig. 1. Effect of amino acid supplement and vitamin levels on the growth of milkfish fry. 1. Corn-gluten meal (CGM); 2. CGM + Lys; 3. CGM + Lys + 5 amino acids; 4. Practical diet with high vitamin; 5. Practical diet.

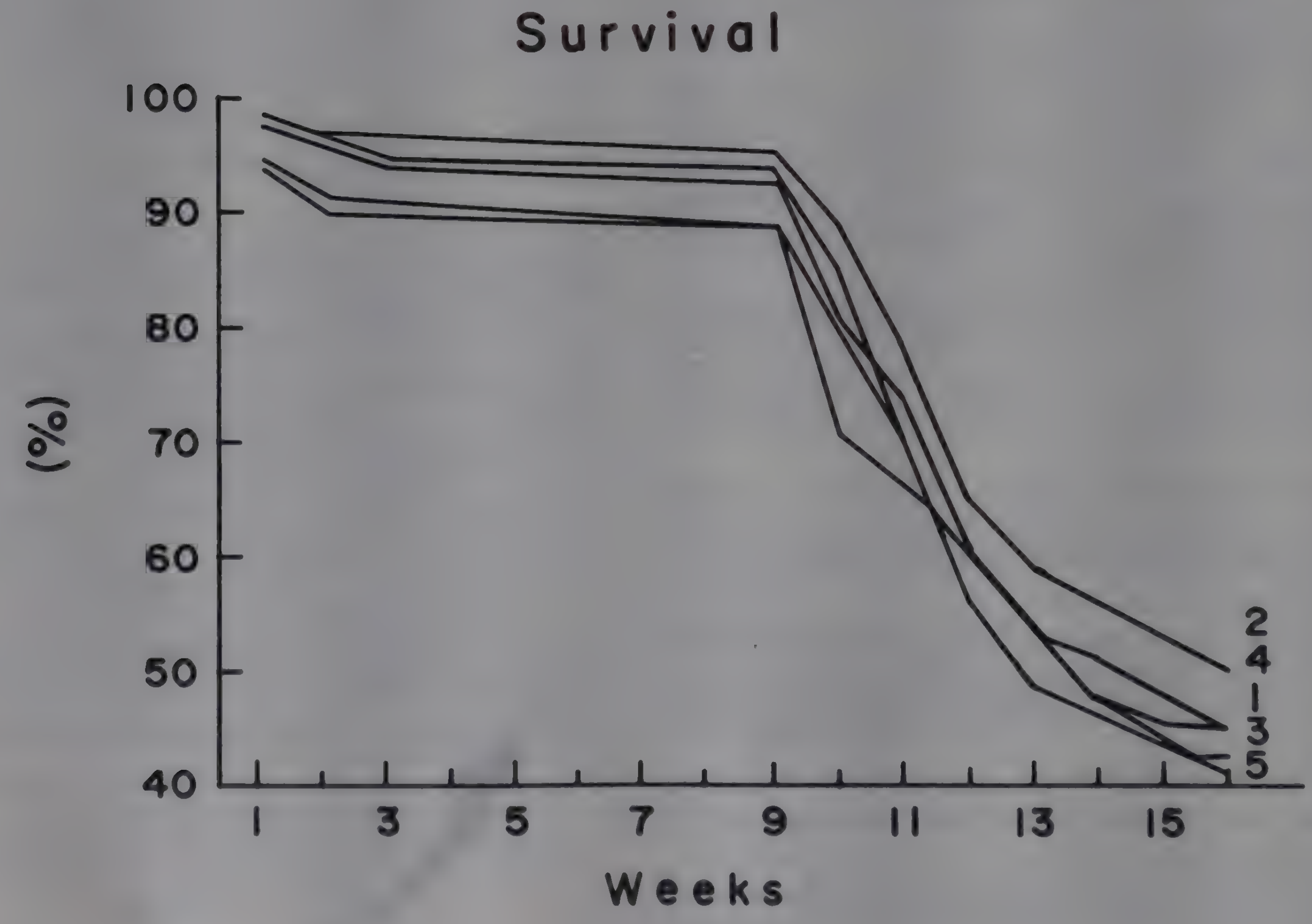

Fig. 2. Percentage survival of milkfish fry, 16-week experimental period. 1. Corn-gluten meal (CGM); 2. CGM + Lys; 3. CGM + Lys + 5 amino acids; 4. Practical diet with high vitamin; 5. Practical diet.

Preliminary Studies of Factors Affecting the Feeding Rhythm of Milkfish (*Chanos chanos* Forskal)*

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Abstract

Milkfish raised in brackishwater fishponds have been observed to be daytime feeders. Feeding activity is characterized by a single peak, which correlates with the intensity of sunlight, the levels of dissolved oxygen (DO) and water temperature and the activity of digestive enzymes. Three experiments were conducted to investigate the effects of DO, artificial feed and light on the feeding rhythm of milkfish. Fish were reared in covered and uncovered circular canvas tanks supplied with water flowing through at different rates from an adjacent pond and were either fed or not fed with artificial feed. Varying flow rates resulted in different DO patterns, with the dawn DO differing by at least 2 ppm. Fish were sampled at regular intervals for 24 hours and feeding index (FI = wt of intestine with content/wt of fish x 100) determined. The three experiments consistently demonstrated feeding behavior patterns: (1) regardless of any of the experimental parameters, peak feeding occurs during the day and the gut is completely devoid of food between 10 p.m. and 2 a.m.; (2) in the presence of both natural and artificial food, natural food is preferred during the light hours while artificial feed is preferred during dark, suggesting preference for photosynthesizing algae; (3) below a certain limiting DO of about 1.5 ppm, milkfish stop feeding; (4) the feeding activity of milkfish is not completely dependent on the presence of light or completely correlated with temperature and DO levels.

Introduction

In the Philippines fish production through aquaculture is dominated by the milkfish industry which contributes some 14% of the total 1.7 x 10^6 t fish produced.

Extensive milkfish culture methods involving the use of pesticides and fertilizers, are widely practiced. Stocking densities range from 2,000 to 3,000/ha. At this level the profitability of culturing milkfish has been decreasing through the years (Smith and Chong 1984) and fish farmers have shown great interest in shifting to the culture of luxury species like shrimps. In Taiwan, the profitability of milkfish farming has been tremendously improved by intensification, which involves feeding milkfish a diet containing 24-29% protein and increased stocking densities (Chen 1981). Intensification, although to a lesser extent, has become a major research thrust in the Philippines (Chiu, in press) to improve the profitability of milkfish culture and to increase the supply of milkfish for the growing population.

To develop a rational feeding program for milkfish, it is important to know the feeding habit of the fish and the factors affecting feeding. Milkfish have been observed to be daytime feeders, characterized by a single, intense peak, shortly after noon, similar to the diel pattern for dissolved oxygen, temperature and intensity of sunlight in ponds (Lin 1969; Chiu and Benitez 1981). It is not clear whether the apparent correlation between the milkfish feeding index and dissolved oxygen, temperature and sunlight is actual or coincidental. It is possible that sunlight alters temperature and photosynthetic rates which at the same time alters dissolved oxygen levels and palatability of the natural food in ponds. The purpose of this investigation was to determine the effect of: (1) dissolved oxygen which is altered by changing water flow rate through a tank, (2) the continuous provision of pelleted diets in addition to natural food and (3) the total exclusion of sunlight from the tank, on the diel pattern of feeding.

Materials and Methods

Experiment 1. Two canvas tanks, 4.57 m in diameter, were stocked with 150 milkfish about 50 g each. The tanks were continuously supplied with water from an adjacent fishpond, flowing at 50 l/min and 10 l/min, respectively. Different flow rates were used to effect two different oxygen regimes in the tanks. The fish were fed 24 hours a day at 10% body weight, using automatic feeders, and were acclimated to the experimental condition for at least ten days. Ten fish were sampled from each tank and from an adjacent pond every four hours for 24 hours starting at 6 p.m. Dissolved oxygen (DO) and temperature were monitored immediately before each sampling. The fish with intestines and contents were immediately weighed and feeding index determined. Feeding index (FI) is defined as: FI = wt of intestine + contents/wt of fish x 100.

Experiment 2. This test further investigaed the effects of DO and pelleted diets on the feeding rhythm and food preference of milkfish, particularly during the transition hours between dark and light. Sampling of eight fish was done at 4, 6 and 8 a.m. and 4, 7 and 10 p.m. Three canvas tanks were stocked with milkfish as in experiment 1. Tanks 1 and 2 were provided with pond water with flow rates of about ten times less (15-35 l/min) than that of tank 3 (250 l/min).

Experiment 3. This study was conducted to determine the effect of light and DO on the feeding activity and food preference of milkfish. Tanks were either exposed or not to sunlight and provided with water at two different flow rates in an experiment with a 2 x 2 factorial design. The tanks with fast flow rate had water flowing from an adjacent pond at 40-80 l/min while those with slow flow rate had water flowing at 20-25 l/min. Two hundred milkfish, averaging 5.4 g, were stocked in each tank. Sampling of seven fish from each tank was done at 6 and 9 p.m., 1, 5, 6 and 10 a.m. and 6 p.m. Temperature and DO were monitored prior to sampling. The method for feeding and evaluating feeding activity were similar to those of the other experiments.

Results

In experiment 1, the diel pattern of DO was very different when water flow varied by at least fivefold in the two tanks. In tank 2, the diel pattern of DO and temperature had a distinct single peak at 2 p.m., coinciding with peak sunlight intensity (Fig. 1). DO in tank 2 was at its lowest levels (below 1 ppm) between 2 and 6 a.m. In contrast, DO was above 3 ppm throughout 24 hours in tank 1.

Fig. 1 shows that in tank 2 the feeding rhythm of milkfish was well correlated with the diel pattern of temperature and oxygen. The intestines of fish in tank 2 were completely empty at 10 p.m., 2 and 6 a.m. At 6 p.m., the feeding index was already drastically reduced. In contrast, the intestines of fish sampled from tank 1 were full at 6 a.m. and empty at 2 a.m. The diel pattern of the feeding index showed a broad peak occurring between 6 a.m. and 2 p.m. Intestinal content during the dark hours was brownish, indicating the dominance of the pelleted feed. During the light hours, intestinal content was greenish, indicating the dominance of natural food, although pelleted feed was continuously provided.

Fish from an adjacent pond were sampled simultaneously with those in the tanks. DO and temperature peaked at 2 p.m. as in tank 2 but low DO levels were above 3 ppm (Fig. 2). As in tank 1, fish were observed to have initiated feeding by 6 a.m. But unlike fish in tank 1, feeding peaked between 10 a.m. and 2 p.m., during which time peak sunlight intensity and photosynthesis occur. Also, unlike that of tank 1, the feeding index was significantly lower at 6 p.m. and the intestines were completely empty by 10 p.m. although DO levels were about 5.5 ppm.

Sampling in experiment 2 was done between light and dark hours. Since coefficients of variation were relatively high, the significance of variance used for each sampling hour were $P \leqslant 0.05$ and $P \leqslant 0.10$ (Table 1). Fish in tank 3 had consistently higher feeding index in the morning samples. The fish fed on artificial feed at 4 and 6 a.m. and shifted to natural food at 8 a.m. DO levels were above 4 ppm during all sampling periods. At the lower flow rate, feeding on a natural food commenced after 6 a.m. The feeding index did not differ significantly whether or not artificial feed was provided for (tanks 1 and 2). DO levels in tank 1 were above 3.5 ppm during all sampling periods but no pellet was provided.

A significantly higher ($P \leqslant 0.05$) feeding index was observed in fish sampled at 7 p.m. from tank 3. Fish provided with pellets in tanks 2 and 3 reflected the dominance of pellets in their intestines. Fish sampled from tank 1 had the lowest feeding index, their intestines containing only remnants of natural food. Although no significant difference ($P > 0.05$) in feeding indices was observed among fish sampled at 10 p.m., fish intestines still contained remnants of the artificial feed, while those of other treatments were empty.

In experiment 3, covering tanks 1 and 3 to eliminate sunlight resulted in difference in temperature of no more than 0.5°C in any of the four tanks. Temperature was lowest at 24.8°C at 5 a.m. and highest at 30.5 at 6 p.m. (Table 2). Tests in tank 3 resulted in a significant difference in the diel DO pattern with minimum DO levels between 5 and 6 a.m. at 1.5 ppm compared with the uncovered tank 2. DO levels in tanks 1 and 4 were above 4 ppm. The feeding indices of fish reared in these tanks were significantly higher ($P \leqslant 0.05$) than those in tanks 2 and 3. Fish in all tanks, except in tank 3, started to feed on the pellets at 6 a.m. Consistent with the earlier experiments, gut analysis of fish at daytime suggests a shift to natural food. A shift back to artificial feed in all treatments is evident at 6 p.m. when natural food is observed in the posterior intestine and feed is in the anterior. Regardless of exposure to sunlight, peak feeding is observed between 10 a.m. and 6 p.m. At 6 p.m. the feeding index of fish in tank 3 was significantly lower ($P \leqslant 0.01$) than those of the others although DO was above 4 ppm.

Discussion

The tanks were supplied with water from an adjacent natural pond so that various biological and chemical parameters could be simulated; specifically, the diel profile of DO and temperature and natural food would abound just as in the pond. In relation to the biomass of fish, DO was apparently the major parameter affected by water changes. Flow rates, defined as slow or fast, had a wide range because of the fluctuating electrical power for the pumps. For the same reason, and because of the varying biomass in the tanks, the DO profile was not clearly defined. Instead, water flow rate was adjusted to attain certain ranges of DO and differences of at least 2 ppm in the early morning DO of tanks exposed to slow and fast flow rates.

The three experiments consistently demonstrated a number of points regarding the feeding habit of milkfish. (1) Regardless of any of the experimental parameters, the whole digestive tract of milkfish is completely devoid of food between 10 p.m. and 2 a.m. which indicates the absence of feeding during this time and even earlier. Even the absence of light in the covered tanks did not change this pattern. (2) In the presence of both natural and artificial food, natural food is preferred from 6 a.m. to 6 p.m. and artificial feed shortly before or after this period. The light hours coincided with the occurrence of photosynthesis. It is possible that photosynthesizing algae is more palatable and therefore preferred. (3) Below a certain limiting DO, 1.5 ppm, milkfish stops feeding. (4) The apparent biological rhythm of feeding is not completely dependent on the presence of light completely correlated with DO and temperature levels. Feeding activity diminishes faster than the decrease of temperature and DO levels after dusk. Moreover, feeding activity can start before dawn and continue after dusk if DO is adequate and artificial feed is provided. These conditions result in feeding characterized by intake over a longer duration.

Various physical and chemical conditions and availability of food affect the amount and fate of food consumed by fishes. An understanding of these factors can provide a rational basis in influencing the fate of food consumed to attain the highest possible growth rate. The effect of oxygen in this experiment is similar to that observed in a study on coho salmon, in that food consumption increased with increasing DO concentration (Warren 1971). In coho salmon, growth rate increased slightly with increasing availability of oxygen, but not as much as food consumption rate because respiration increased primarily as a result of increased specific dynamic action. Excessively high DO levels, on the other hand, has been found to depress growth (Warren 1971). If this phenomenon occurs in milkfish culture, the removal of highly supersaturated oxygen, which occurs at the peak of the day with a plankton bloom, may significantly decrease production. This may be reduced by using equipment like paddlewheel aerators, which can be simultaneously used to maintain DO above limiting levels before dawn. This study suggests that changes in DO levels resulting from such interventions and the availability of food can change the pattern of feeding. An experiment needs to be conducted to determine if such management practices improve the efficiency of food utilization and consequently fish growth. For the fish farmers, it would be of tremendous interest to determine the economic advantage from the energy inputs invested in feeding and in certain water management practices.

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*USAID Aquaculture CRSP Contribution No. 86:03.

Table 1. Effect of artificial feed and water flow rate on dissolved oxygen and on the feeding rhythm of milkfish (Experiment 2).

Tank no.	Sampling time (hr)	Water temperature (°C)	Flow rate[1]	Feed	DO (ppm)	Feeding index[2]	Intestinal contents[3]
1	4 a.m.	26.5	Slow	None	3.6	2.60 ± 0.32	Empty
2			Slow	Feed	1.7	2.60 ± 0.37	Empty
3			Fast	Feed	4.2	3.70 ± 0.36**	Brownish matter in posterior
1	6 a.m.	25.9	Slow	None	3.6	2.67 ± 0.42	1/2 empty
2			Slow	Feed	1.6	2.83 ± 0.36	Empty
3			Fast	Feed	4.4	3.83 ± 0.54	1/2 full (brown)
1	8 a.m.	26.1	Slow	None	3.6	2.36 ± 0.34	1/4 full (green)
2			Slow	Feed	2.1	3.65 ± 0.42	1/4 full (green)
3			Fast	Feed	4.8	4.31 ± 0.91*	1/2 full (green) in anterior, brown in posterior
1	4 p.m.	28.1	Slow	None	6.1	4.97 ± 1.14	3/4 full (green) in posterior
2			Slow	Feed	4.5	5.35 ± 0.79	Full (green)
3			Fast	Feed	7.1	5.63 ± 0.52	Full (brown)
1	7 p.m.	28.2	Slow	None	6.1	2.66 ± 0.35	1/4 full (green)
2			Slow	Feed	5.1	3.66 ± 0.34	Brownish matter in anterior
3			Fast	Feed	6.0	5.01 ± 0.96**	3/4 full (brown)
1	10 p.m.	27.2	Slow	None	5.0	2.54 – 0.39	Empty
2			Slow	Feed	3.4	2.37 – 0.17	Empty
3			Fast	Feed	4.9	2.51 – 0.41	Yellowish-brown semi-fluid matter

[1] Slow flow rate: 3.8-7 gpm; Fast flow rate: 67 gpm.

[2] Values represent the mean ± SEM of ten fish; values with asterisks are significantly higher than values of other treatments of the same sampling period (** – $p < 0.05$; * – $p < 0.10$). The rest are not significantly different.

[3] Visual observation on more than 50% of the samples. Brownish color suggests pelleted feed; greenish color suggest natural food.

Table 2. Effect of photoperiod, flow rate, temperature and dissolved oxygen on the feeding rhythm of milkfish (Experiment 3).

Tank no.	Time (hr)	Cover	Flow rate[1]	Temperature (°C)	DO (ppm)	Feeding index[2]	Intestinal contents[3]
1	6 p.m.	Covered	Fast	28.8	5.9	6.11 ± 0.33^a	Full (brown in anterior, green posterior)
2		Not covered	Slow	28.3	5.0	6.03 ± 0.70^a	Full (brown anterior, (green posterior)
3		Covered	Slow	28.4	3.0	5.76 ± 0.58^a	Full (brown anterior, green posterior)
4		Not covered	Fast	28.4	6.0	6.77 ± 0.69^a	Full (brown anterior, green posterior)
1	9 p.m.	Covered	Fast	27.3	5.0	3.89 ± 0.41^a	1/4 full (brown)
2		Not covered	Slow	27.2	3.0	4.92 ± 0.50^a	1/4 full (brown)
3		Covered	Slow	27.4	2.0	6.59 ± 0.72^b	1/8 full (brown)
4		Not covered	Fast	27.4	5.3	4.97 ± 0.38^a	1/8 full (brown)
1	1 a.m.	Covered	Fast	26.3	4.8	3.10 ± 0.24^a	Empty
2		Not covered	Slow	25.9	2.7	2.90 ± 0.18^a	Empty
3		Covered	Slow	26.4	1.6	2.51 ± 0.31^a	Empty
4		Not covered	Fast	26.2	5.2	3.38 ± 0.39^a	Empty
1	5 a.m.	Covered	Fast	25.4	4.8	3.39 ± 0.12^b	Empty
2		Not covered	Slow	24.8	2.5	2.05 ± 0.19^a	Empty
3		Covered	Slow	26.1	1.5	2.44 ± 0.33^a	Empty
4		Not covered	Fast	25.2	5.2	3.31 ± 0.44^b	Empty
1	6 a.m.	Covered	Fast	27.5	4.8	4.35 ± 0.30^b	3/4 full (brown)
2		Not covered	Slow	27.2	3.0	3.71 ± 0.33^a	1/4 full (brown)
3		Covered	Slow	27.8	1.5	2.68 ± 0.64^a	Empty
4		Not covered	Fast	27.2	3.8	4.49 ± 0.50^b	Full (brown)
1	10 a.m.	Covered	Fast	28.8	5.1	4.47 ± 0.80^a	1/4 full (green)
2		Not covered	Slow	29.0	4.4	3.79 ± 0.25^a	1/2 full (green)
3		Covered	Slow	28.5	1.8	3.66 ± 0.56^a	1/4 full (green)
4		Not covered	Fast	29.0	5.4	5.43 ± 1.07^a	Full (green)
1	6 p.m.	Covered	Fast	30.5	6.2	6.74 ± 0.61^b	Full (brown)
2		Not covered	Slow	30.2	5.8	6.72 ± 0.66^b	Full (green)
3		Covered	Slow	30.2	4.9	4.19 ± 0.41^a	Full (brown anterior, green posterior)
4		Not covered	Fast	30.0	6.2	6.96 ± 0.69^b	Full (brown anterior, green posterior)

[1] Fast: 11-20 gpm; slow: 5.2-6 gpm.

[2] Values represent mean ± SEM of seven fish; values with different letters are significantly higher than other treatments of the same sampling period.

[3] Visual observation on more than 50% of samples.

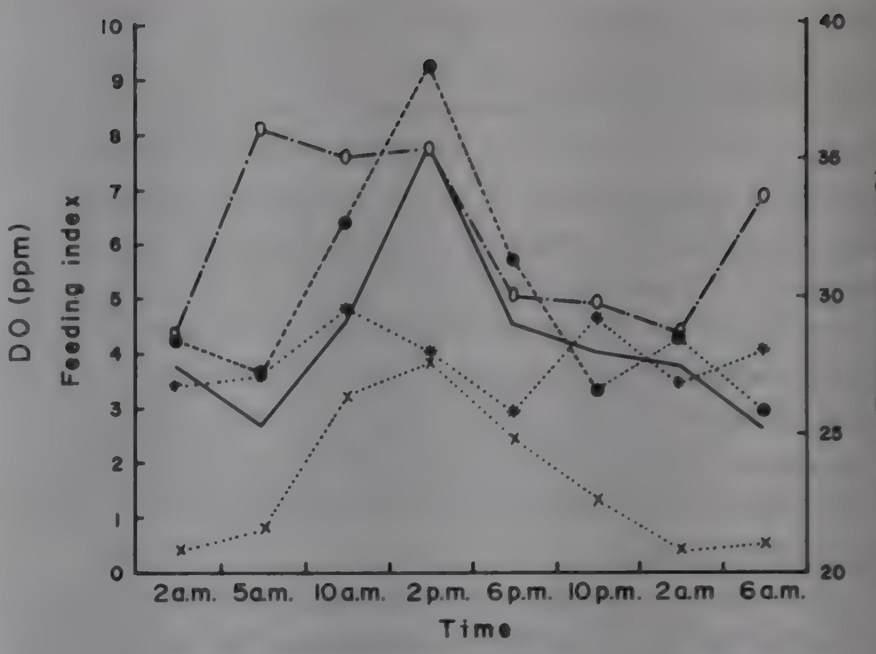

Fig. 1. Effect of water flow rate in tanks on the diurnal pattern of dissolved oxygen, water temperature and feeding index of milkfish. * dissolved oxygen; ○ feeding index high flow rate; X dissolved oxygen low flow rate; ● feeding index low flow rate; — temperature.

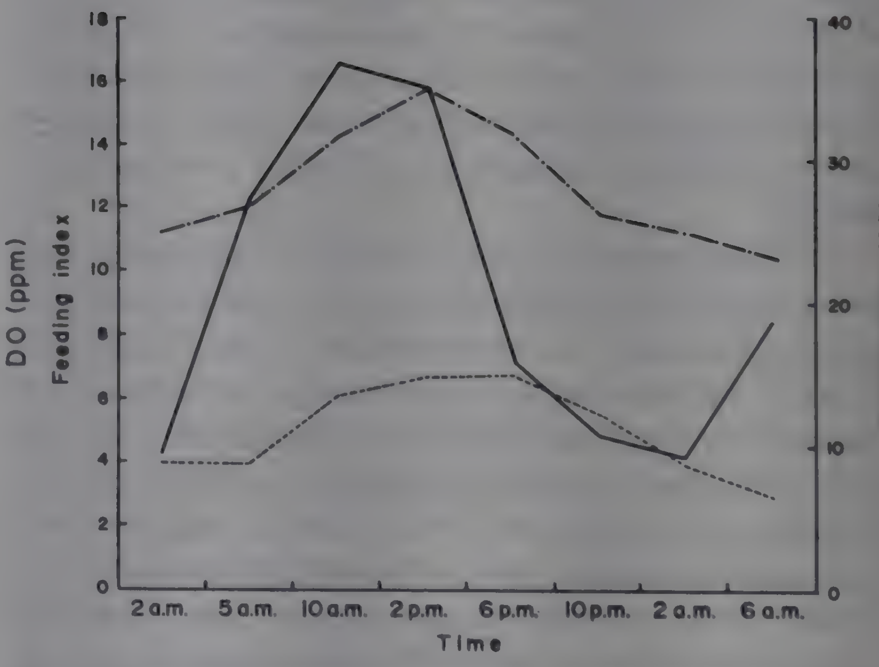

Fig. 2. The diurnal pattern of dissolved oxygen, water temperature and feeding index of milkfish in a brackishwater pond. — — — dissolved oxygen; ——— feeding index; — . — temperature.

Ingestion Rate of Brine Shrimp Nauplii by *Metapenaeus ensis:* Effects of Food Concentration and Starvation

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Abstract

Newly hatched brine shrimp nauplii from decapsulated cysts were fed to *Metapenaeus ensis* from mysis to postlarva (PL) 14 in various food concentrations. The daily ingestion rate was determined every other day by measuring the decrease in number of nauplii in experimental vessels. The ingestion rate of postlarvae was found to increase with food concentration up to a saturated maximum rate. The maximum ingestion rate was not evident in the mysis stage and increased gradually from PL 2 to PL 14. The weight-specific maximum ingestion rate appeared to stabilize after PL 6. The results give an estimate of the daily optimal food concentration for cultured shrimp. The effect of starvation on ingestion rate was investigated by feeding the shrimp every other day. The ingestion rate of the starved shrimp was not significantly different from that of the shrimp fed daily. Starvation, however, increased mortality, which may be due to cannibalism.

Introduction

Successful rearing of penaeid shrimp larvae depends on supplying suitable live foods to the shrimp as development proceeds. Cultured shrimp at their mysis and early postlarval stages are usually fed freshly hatched nauplii of brine shrimp (Liao et al. 1983). However, the use of this expensive food source can seldom be optimized as little is known about the feeding behavior of shrimp on artemia nauplii. A number of reports on this aspect of penaeid shrimp have appeared in recent years (Gopalakrishnan 1976; Emmerson 1984; Yúfera et al. 1984). The present work investigated the effects of food concentration and starvation on the ingestion rate of artemia nauplii by the shrimp *Metapenaeus ensis* currently cultured in Southeast Asia.

Materials and Methods

Gravid *Metapenaeus ensis* females were obtained from local fishermen and maintained in individual 500-l fiberglass tanks containing well aerated filtered seawater treated with 10 mg/l ethylene-diamino tetra-acetic acid, disodium salt (EDTA). The shrimp spawned in the early hours of the following day. After a single spawning, the spawner was removed to avoid a second spawning and to obtain a larval stock with synchronized development.

Nauplii from a single spawner were stocked in three 500-l tanks at a density of about 30,000 larvae/tank. Larvae from nauplius 6 to mysis 2 were fed a mixture of BP diet ("Nippai" brand at 2 mg/l) and unicellular algae, which consisted of the blue-green alga *Microcystis* (species unknown) and mixed diatoms. From mysis 3 to PL 14, the shrimp were fed freshly hatched artemia nauplii from dry cysts ("San Francisco Bay" brand). Shrimp in two of the tanks were fed a ration gradually increasing from 10 to 100 nauplii/larva/day. This ration was based on results of preliminary feeding experiments. Shrimp in the third tank were fed nauplii at the same ration every other day. The ingestion rate of animals in this tank was determined on the day after they had been starved. Experiments on daily-fed animals from one of the previous tanks were run simultaneously. Daily-fed animals in the other tank were not taken for any experiments and acted as control for the effect of handling on shrimp mortality. The survival rate of the shrimp from mysis to postlarva 14 was estimated in each tank at the end of the culture period. Throughout the culture period, seawater in each tank was renewed every other day. The range of temperature and salinity in the tanks was 26-32°C and 25-33 ppt, respectively.

Daily ingestion rate of shrimp on artemia nauplii was determined by estimating the decrease in density of nauplii in experimental vessels containing shrimp taken from the culture tanks. Artemia nauplii hatched from decapsulated cysts were used as prey as the nauplii could easily be separated from unhatched and empty cysts so that a reasonably constant concentration of nauplii could be maintained in control vessels. The decapsulation cysts were prepared with a method modified from Sorgeloos et al. (1977) and Bruggeman et al. (1980). Aliquots of the nauplii stock were diluted to 1 l with finely filtered seawater to give the desired food concentrations, range 2-30 nauplii/ml. This initial food concentration, C_i, in each experimental vessel was determined by counting the number of nauplii in 10 x 5 ml samples. One hundred shrimp were then transferred from the culture tanks to each vessel to give a density of 100 shrimp/l. Two controls, containing artemia nauplii but no shrimp, were run simultaneously.

After 24 hours, the concentration of nauplii, C_t, was measured with the same sampling method. Shrimp were returned to the original culture tanks after experiments. The decrease in concentration was corrected for the change in nauplii number in shrimp-free controls. This correction, which was mainly due to sampling error, seldom exceeded 10%.

Ingestion rate, I, was calculated from the equation (Paffenhöfer, 1971):

$$I = V \frac{(C_t - C_i)}{n \cdot t}$$

where $(C_t - C_i)$ is the decrease in concentration of nauplii within the experimental period, t; V is the volume of the experimental vessel; and n is the number of shrimp.

Results and Discussion

The daily ingestion rate as a function of food concentration for shrimp at various developmental stages is shown in Fig. 1. The ingestion rate by mysis 3 was low, below 20 nauplii/larva/day. In later stages, the ingestion rate increased with food concentration up to a maximum which remained relatively constant with further increase in food concentration. This maximum ingestion rate gradually stepped up as the postlarva aged. The rate in nauplii/larva/day was about 10 for mysis 3, and increased by 10 to 15 every two days to 100 for PL 14. This gives an estimate of the optimal ration for these stages at a stocking density of 100 shrimp/l.

Fig. 1 also shows that at all food concentrations tested, ingestion rate of starved shrimp was essentially similar to that of daily-fed shrimp. Starvation, however, might affect survival of shrimp in the culture tank. The percentages of survival from mysis to PL 14 were 53 in the tank where no animals were taken for experiments, 68 in the tank where daily-fed animals were taken for experiments and 41 in the tank where animals were starved. Although these estimates are subject to sampling error, it seems that feeding shrimp every other day reduces the survival of shrimp, showing that daily feeding with artemia nauplii is necessary.

A possible explanation for an absence of starvation effect on ingestion is the competition between the shrimp in capturing artemia nauplii at low food concentration. The less competitive shrimp might starve and die in the culture tank, so that they could not be selected for feeding experiments. It is also possible that the weaker shrimp might have been eaten by the others on the day they were not fed. The death of shrimp due to either food deficiency or cannibalism could account for the increase in mortality in the culture tank. In any case, the animals taken for feeding studies might have not been starved before the experiments and thus exhibited an ingestion rate similar to those fed daily.

The maximum ingestion rate per unit body weight of shrimp is shown in Fig. 2. This weight specific ingestion rate appeared to increase with development from mysis 3 to about PL 6, after which the rate stabilized. The data imply that stages before PL 6 ingested less nauplii per unit body weight than the later stages. It is likely that these earlier stages may rely on microalgae as part of their diet when gradually shifted to raptorial feeding on zooplankton, as illustrated by the rapid increase in ingestion rate on artemia nauplii during this period.

Acknowledgment

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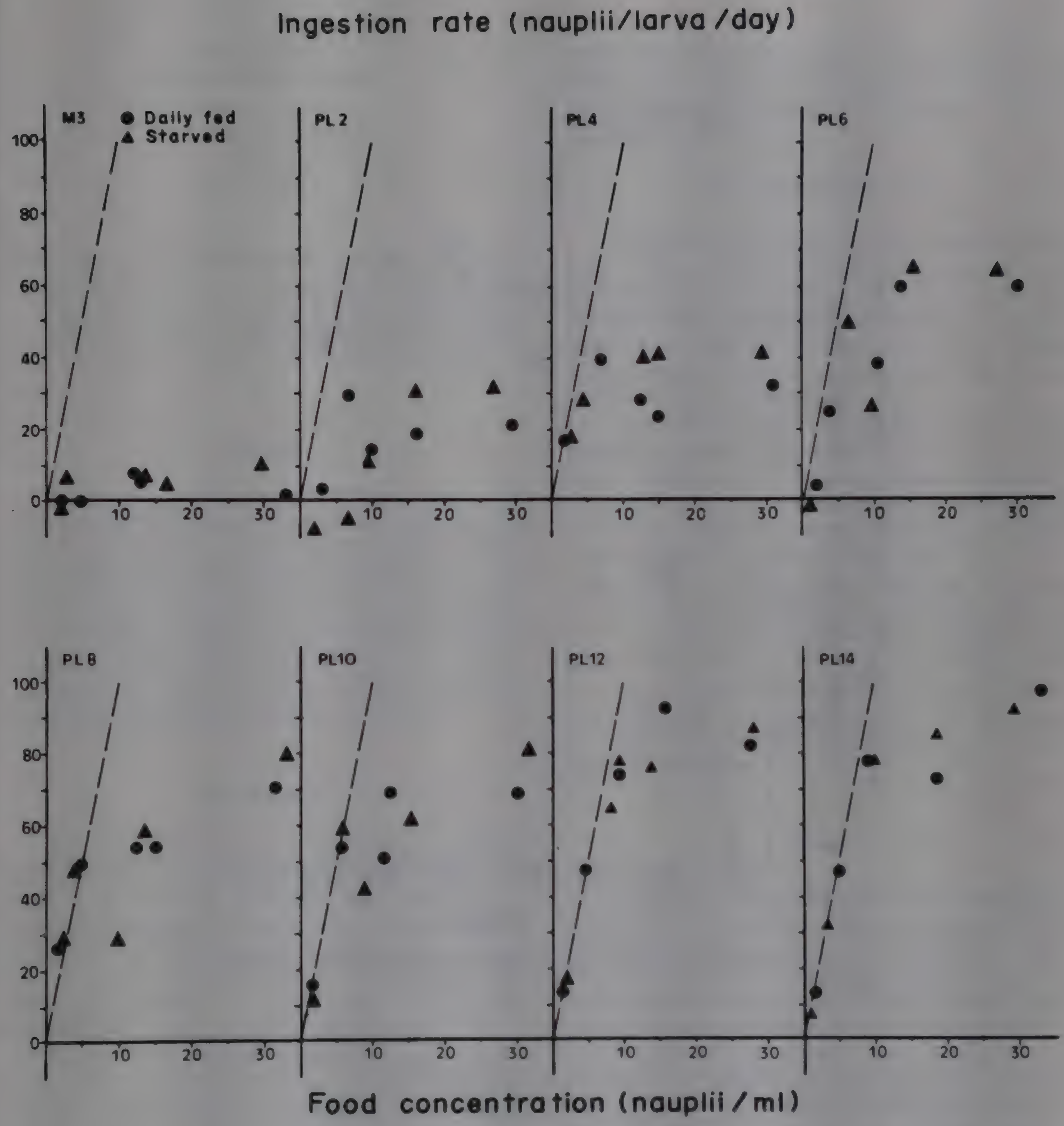

Fig. 1. Effect of artemia concentration on ingestion rate of *Metapenaeus ensis* fed daily or every other day. The broken lines represent complete clearance of nauplii provided.

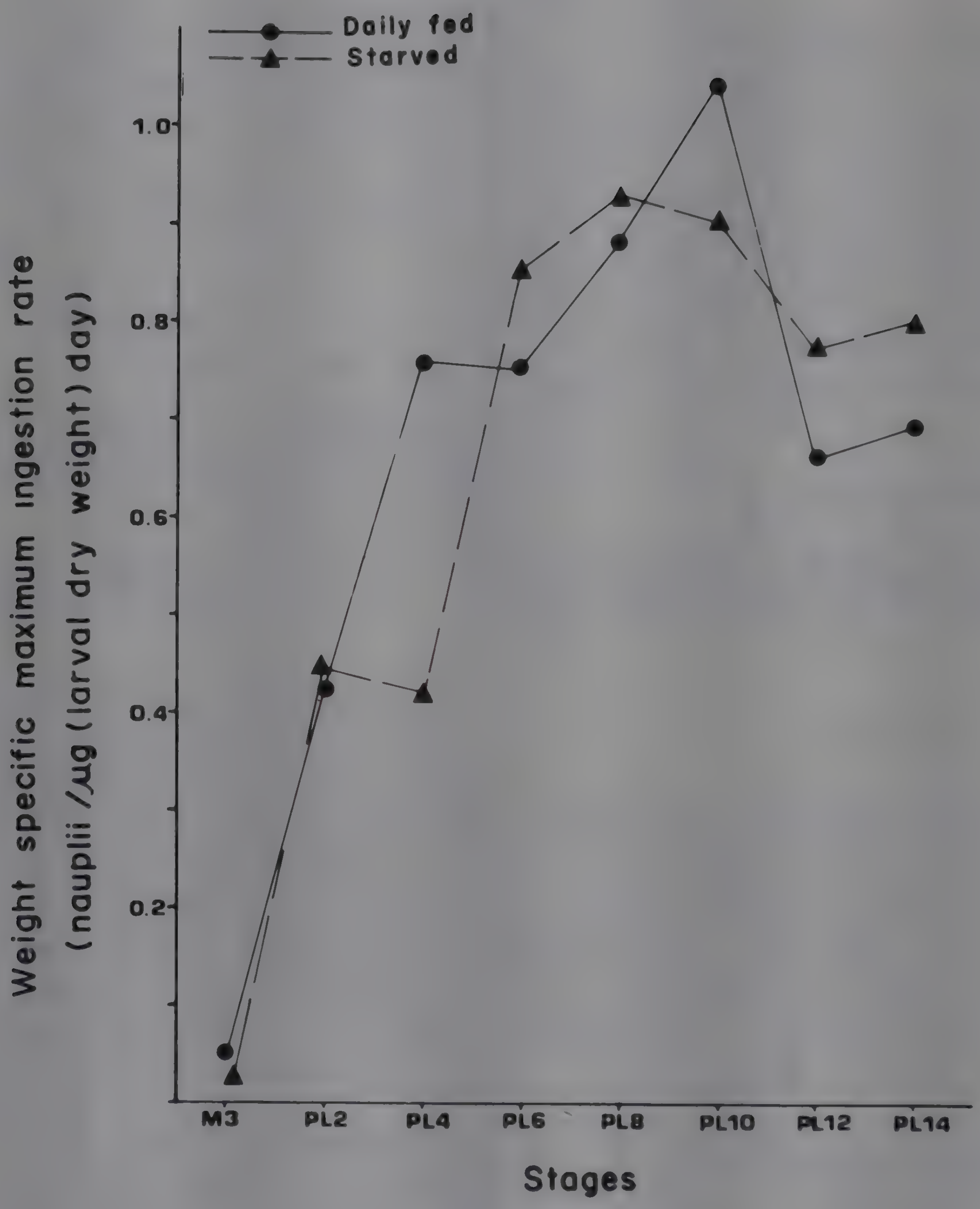

Fig. 2. Weight-specific maximum ingestion rate of *Metapenaeus ensis* feeding on artemia.

Food, Feeding and Fecundity of the Giant Freshwater Prawn *Macrobrachium rosenbergii* from Natural Habitats in Sri Lanka

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Abstract

The food, feeding and fecundity of *M. rosenbergii* (De Man) were studied from specimens caught from the three main *Macrobrachium* fishery areas in Sri Lanka. The food of the prawns of all size groups studied consisted mainly of detritus, plant and animal matter of which detritus formed the main food component. Of the food 20-40% was of animal matter while 10-20% was of plant matter. Sand particles, possibly ingested accidentally, formed about 3-5%. Some selective feeding was observed in different size groups. The fecundity ranged from 19,000 to 137,000 in prawns measuring 12-31 cm. Fecundity showed a linear relationship to body length, carapace length and body weight. The egg diameter enlarged with increase in body length of the spawner.

Introduction

Macrobrachium rosenbergii (De Man) is a common freshwater prawn species in the Indo-Pacific region. This prawn is an esteemed food item and commands an excellent market price. It inhabits both brackishwater and freshwater areas in the lowland. It is becoming rare now due to overexploitation, effects of pollution and conflicting demands of other water users, thus necessitating its restocking even in natural waters.

Because of its potential in aquaculture most of the recent work on this prawn has been confined to studies on large-scale farming. However, knowledge of the biology of natural populations and the fishery of this prawn in the Asian region is meager and is limited to a few studies such as those of Rajyalakshmi (1961), Rao (1967) and Patra (1976).

Little or no work has been done on the biology and the fishery of this prawn in Sri Lanka except for Costa (1979). The present investigation was carried out to obtain information on the food, feeding habits and fecundity of natural populations of this prawn in Sri Lanka.

Materials and Methods

The study on food habits was performed on animals collected from three different areas in Sri Lanka: Bolgoda lake (Panadura area), Dandugam Oya (Katunayake area) and Lunu Oya (Chilaw area). Immediately on capture the prawns were killed by injecting them with 5% formalin with a syringe through the mouth and anus. The stomachs were preserved in 10% formalin.

In the laboratory the excised stomachs were weighed. Volumetric estimates (Windell 1971) were made for the major categories of stomach content components categorized under fine particulate matter, macroflora and macrofauna. Individual components of the macrofauna were identified to the nearest possible taxon.

Fecundity studies were carried out on berried females taken from the collecting sites. In the laboratory the total weight, total length and carapace length of each prawn were measured. The eggs of individual prawns were stored in tubes containing Gilson's fluid. Fecundity was determined by volumetric methods. The diameter of the eggs was measured under a microscope with an eyepiece micrometer.

Results

Volumetric analysis (%V) of the macrofaunal dietary components of various size categories of prawns collected from the three different habitats is presented in Table 1. The distribution pattern of the food components of prawns from all the three habitats is given in Fig. 1. River detritus formed the major components in all four size groups.

Macrofaunal components were present in the majority of the stomachs examined from each of the three areas sampled. The volumetric contribution was 23.1, 36.4, 25.0 and 32.7%, respectively, for size groups of prawns sampled from all the areas (Fig. 1). Differences were less clearly defined between the size groups. The macrofaunal components consisted mainly of crustacean appendages, remains of both aquatic insects and insect larvae, small worms, scales of fish and gastropod fragments. It was also interesting to note that the smallest prawns collected did not contain even a single fragment of gastropods while molluscan fragments were frequently encountered in the stomach contents of the bigger size groups.

The macro- and microfloral components formed the third largest food components. These consisted of pieces of leaves and stems of macrophytes, benthic and planktonic algae and plant seeds. In some, pieces of *Salvinia, Eichhornia* and *Chara* were clearly dicernible. Small amounts of sand were found in almost all the stomachs examined.

The fecundity of 86 prawns of varying sizes from the three localities was determined. The mean fecundity of specimens of various size groups from 12 to 31 cm, was 18,933-136,727 (Table 2). The fecundity was observed to increase with body length. The maximum size of berried females encountered in this study was 28.9 cm with a fecundity of about 170,000.

Table 3 shows the correlation coefficients, regression equations and the significance of correlation of fecundity with total length, carapace length, body weight and gonad weight. There is a significant linear relationship of fecundity to body length, carapace length, body weight and gonad weight.

The egg diameter distribution of length groups is shown in Fig. 2. The diameter of the eggs ranged from 0.455 mm to 0.665 mm. There was an increase in the diameter of the eggs with increase in size of the prawns.

Discussion

Examination of stomach contents showed that organic matter is the major food of *M. rosenbergii* as it formed about 40-45% of the total food taken by the prawns in all the areas studied. These results confirm the observations of Darnell (1974) and Fenchel and Jorgensen (1977). These workers observed that *M. rosenbergii* which live in the epibenthos like other decapod crustaceans, derive their major nutrition from their environment through various forms of detrital ingestion. According to Barnes (1974) detritus is a major food source of estuarine animals. The importance of detritus in riverine and estuarine ecosystems results from the microbes associating with the dead organic particles (Odum and Heald 1972). Feeding anatomy and habits lend themselves well to this sort of detritivory (Patwardhan 1935).

Specimens of *M. rosenbergii* collected from all three areas studied contained varied amounts of detritus (44%-55%), animal matter (21%-36%) and plant matter (11%-24%). Rao (1967) who examined stomach contents of *M. rosenbergii* in the field observed that this species ingests a wide variety of food of both plant and animal origin as well as detritus thereby functioning as a primary consumer, secondary consumer and detrivore in the aquatic system. Ling (1969) and Malecha et al. (1981) indicated that *M. rosenbergii* could be classified as an omnivore because of its ready acceptance of a variety of food types which include worms, insect larvae, adult aquatic insects, small molluscs, other crustaceans, fish, grain seeds, nuts, fruits, algae, leaves and stems of aquatic plants.

The amount of sand contained in the stomachs varied in specimens collected from different areas and showed an average range of 3.7-6%. The sand particles may have been ingested accidentally with other food items.

Among the macrofaunal elements in the food examined in this study were molluscan fragments, mostly observed in the larger specimens. The bottom-inhabiting gastropods undoubtedly fall easy prey to the larger prawns in search of food. Subramanyam (1963) indicated that gastropods and bivalves formed about 11-90% of the food of *Metapenaeus affinis*, with the older shrimp appearing to prefer mainly a molluscan diet. George (1972), who analyzed the food contents of these prawns in the backwaters of Cochin, states that as size increased selective feeding becomes more evident.

A general increase in the animal matter and a decrease in the amount of plant matter observed in larger prawns may be a reflection of their relative preference for animal food items. As George (1972) and Ling (1969) have remarked, the nature of the food ingested will be dependent on relative abundance of types of food present in the habitat.

Studies on the fecundity of wild *M. rosenbergii* have shown a linear relationship to body length, carapace length, body weight and gonad weight. These conform to the observations made by Patra (1976) on specimens from Bangladesh. Similar linear relationships between the length of the female and the number of eggs have been described in other *Macrobrachium* species, such as *M. rude* (Shakuntala 1976). A linear relationship between the number of eggs carried by berried females and carapace length has also been shown in *M. dayanum, M. lamarrei* and *M. lanceifrons* (Rasalan et al. 1980).

The mean fecundity of wild *M. rosenbergii* of 12-31 cm length varied from 20,000 to 137,000. This number is similar to the values reported by Patra (1976) who estimated the mean fecundity of 20 specimens of *M. rosenbergii* from Bangladesh to be around 130,000 with a range of 54,000-276,000 depending on the size and condition of the individual.

Ling (1969) observed that a female *M. rosenbergii* weighing about 80 g and 18 cm in length can produce about 60,000-100,000 eggs. The wild specimens from Sri Lanka measuring 17-18.9 cm and weighing 75-85 g produced around 36,000-75,000 eggs with a mean fecundity of 59,422. Chacko (1955) showed that in prawns the number of eggs extruded at a time depends mainly on the size of the individual. It was estimated by Rajyalakshmi (1961) that a female 20 cm in length produces around 111,400 eggs while a female 13.6 cm in length produces only 7,000 eggs.

The largest egg diameter observed was 0.665 mm. Ling (1969), however, described the maximum egg diameter of *M. rosenbergii* as only 0.570 mm.

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Table 1. Macrofaunal components in the stomach of *M. rosenbergii* of various size categories.

Prawn size category (cm)	Macrofaunal component % C	P	K	Components	Chilaw %	Panadura %	Katunayake %
10.0-14.9	20	26	23	Crustacean appendages	11.3	15.9	13.9
				Insect remains	3.4	3.1	2.1
				Gastropod fragments			
				Small worms	2.1	2.0	3.2
				Fish scales	3.2	1.5	4.2
15.0-19.9	31	36	40	Crustacean appendages	18.7	29.7	30.2
				Insect remains	5.3	3.4	4.0
				Gastropod fragments			
				Small worms	3.5	1.7	2.6
				Fish scales	4.2	2.1	3.8
20.0-24.9	30	29	16	Crustacean appendages	7.9	16.3	5.4
				Insect remains	3.1	2.6	1.4
				Gastropod fragments	14.2	7.2	5.1
				Small worms	1.8		2.0
				Fish scales	3.0	3.0	2.1
25.0-29.9	26	30	40	Crustacean appendages	10.5	9.8	11.8
				Insect remains	4.2	3.4	5.4
				Gastropod fragments	7.9	16.3	17.6
				Small worms			2.3
				Fish scales	3.4	2.4	3.1

Table 2. Range and mean fecundity in different size groups of *Macrobrachium rosenbergii*.

Length group (cm)	Number of observations	Fecundity range	Mean fecundity
13.0-14.9	10	3,431 – 51,600	18,933
15.0-16.9	16	7,968 – 59,250	29,185
17.0-18.9	11	36,000 – 75,400	59,422
19.0-20.9	10	48,436 – 84,250	62,782
21.0-22.9	8	45,800 – 106,875	74,027
23.0-24.9	11	50,932 – 130,560	83,487
25.0-26.9	10	107,040 – 163,500	130,317
27.0-28.9	10	44,950 – 170,350	136,727

Table 3. Correlation coefficient, regression equation and significance of correlation of fecundity with total length, carapace length, body weight and gonad weight of *M. rosenbergii*.

Relationship	Correlation coefficient	Regression equation	Significance at 5% level
1. Fecundity (y) and total length (x)	0.80196	$y = 6510.25\,x - 64442.27$	S
2. Fecundity (y) and carapace length (x)	0.81494	$y = 26371.737\,x - 58006.16$	S
3. Fecundity (y) and body weight (x)	0.8428	$y = 562.38\,x + 23351.78$	S
4. Fecundity (y) and gonad weight (x)	0.9332	$y = 5270.74\,x + 21100.9$	S

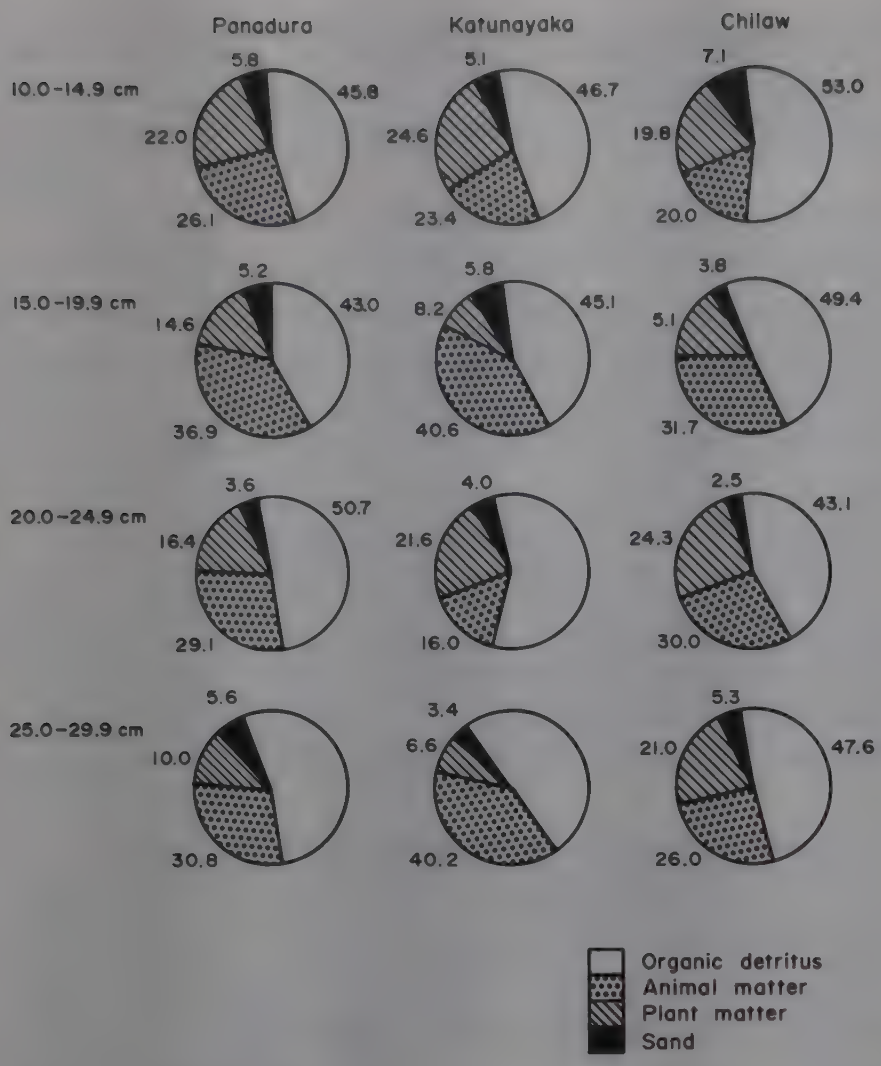

Fig. 1. The main food items of different length groups of *M. rosenbergii* caught from the Panadura, Katunayake and Chilaw areas.

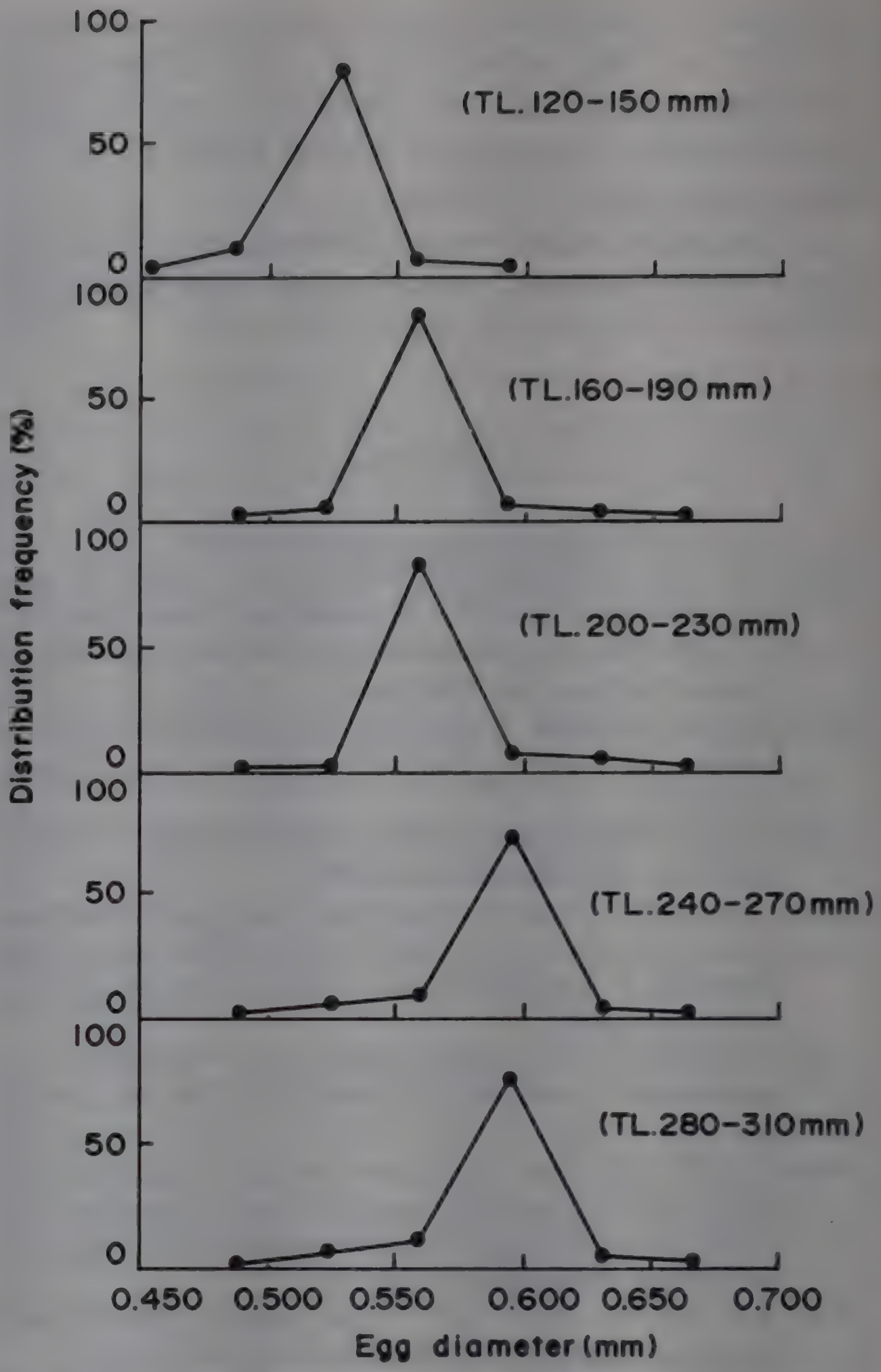

Fig. 2. Relationship between egg diameter of different length groups and the distribution frequency (%) of *M. rosenbergii.*

Optimum Ration and Feeding Frequency in *Oreochromis niloticus* Young

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Abstract

Experiments were designed to evaluate the optimum ration and feeding frequency for *Oreochromis niloticus* young of four size groups. The mean starting weights of the experimental groups ranged from 0.87-1.07 g (Gr. I), 3.23-3.64 g (Gr. II) and 6.28-6.84 g (Gr. III) and 16-17 g (Gr. IV). All experiments were carried out for 70 days in closed, recirculating systems, and as far as possible for each variable, in triplicate. The composition of the test diet was 30.8% protein, 17.48% total lipid and 16.5% ash and 19.6k j/g of energy.

The performance of the fish was assessed on the basis of the specific growth rate, food conversion ratio and protein efficiency ratio. Results indicate that the optimal ration as well as the optimal feeding frequency differs between size groups. For example, in Gr. I the highest SGR was observed when on a ration of 4% body weight/day while in Gr. III which were fed a ration of 3% body weight/day but at different frequencies there was no apparent difference in the performance.

Introduction

Studies on finfish nutrition have been directed mostly towards developing low-cost feeds, especially by substitution of the animal protein ingredients with plant protein substitutes (Cruz and Laudencia 1977; Jackson et al. 1982; Viola and Arieli 1982). In aquaculture the feed cost is often the highest recurring cost. Apart from providing a suitable diet and reducing the ingredient cost, proper husbandry practices also could provide a considerable saving (De Silva 1985). It is known that in poultry and mammals proper husbandry techniques have been responsible, in part at least, in making the enterprises cost-effective.

This paper presents an evaluation of the optimal ration and feeding frequency in four size groups on *Oreochromis niloticus* (L.) young.

Materials and Methods

O. niloticus bred in the outdoor pond of the University of Ruhuna were studied. Each experimental systems except one, consisted of circular tanks of 30 cm diameter and 20 cm depth with a central outflow leding into an intermediate tank of capacity 15 l connected to two reservoir tanks (150 l) with a biological filter. Water was pumped from the reservoir tanks into the experimental tanks by Eheim pumps at a minimum rate of 0.5 l/min. Further details are given by De Silva and Perera (1985). One system consisted of fiberglass tanks of 165-l capacity and was used for the largest size group. In these tanks there was no flow-through system and 1/3 of the water was replenished daily.

All experimental tanks were provided with continuous aeration and a 12:12-hours light:dark cycle was maintained. In all the experiments fish were weighed individually, after anesthetization at the commencement and the termination of each experiment after 70 days. In addition the total weight of all the fish was obtained fortnightly. All experiments were carried out at 26 to 28°C.

The composition of the experimental diet is given in Table I. The particle size of the diet varied from 400μm to 3 mm particles, depending on the size of experimental fish. The details of the individual experiments performed on each size group of *O. niloticus* young are summarized in Table 2. All experiments, unless otherwise stated, were carried out in triplicate and the results are expressed as the grand mean of the replicates.

Results

The daily food consumption in *O. niloticus* young of three size groups, fed *ad libitum* but at different frequencies, is given in Table 3 and Fig. 1. The daily consumption varied considerably within a group, as well as between groups, when fed at different frequencies. In general a day of high intake was followed by a day of low intake, irrespective of the number of times fed. The weekly mean intakes, on the other hand, were considerably high at the commencement of an experiment but the variability appeared to be reduced after a fortnight or so. Also, the weekly variability was less at lower feeding frequencies.

The overall mean intake was mostly independent of the feeding frequency with one exception in that the intake differed significantly when fish were fed once a day as against four times a day. Also the mean intake per day decreased markedly with increasing body weight initially, and thereafter the decrease was of smaller magnitude. The mean consumption was significantly related to the mean wet body weight (Fig. 2) and the mathematical relationship between these two parameters is expressed by the equation:

$$Y = 2.378 + \frac{1.849}{X^2} \quad (F = 3{,}037.58;\ SE = 0.81;\ p < 0.01)$$

where Y = mean consumption per day as a percentage of the body weight and X = mean body weight in g.

For clarity and convenience it is more appropriate to consider the growth performance in relation to: (a) the feeding frequency and (b) the ration size.

The mean fortnightly weights of *O. niloticus* young of starting weight 0.8760-1.0810 g and 6.8464 g are shown in Fig. 3. The rates of growth measured as the per cent average daily gain (% ADG) of the different experimental groups are given in Table 4. Of the *O. niloticus* young fed *ad libitum* the best growth performance was observed in those fed four times a day (size Grs. I and III), but those fed a predetermined ration once or twice a day performed best (size Gr. III).

The mean fortnightly increases in weight of *O. niloticus* young of two size groups (Gr. II-3.0374 to 3.3609 g and Gr. IV-14.4 to 17.8 g) fed twice a day but at predetermined rations or *ad libitum* are shown in Fig. 4. In size Gr. II *O. niloticus* fed 6% body weight per day grew best, and those fed *ad libitum* fared better than those given only a 2% ration. The best %ADG was 1.66.

In general there was a decrease in the %ADG as size increased. In size Gr. II the treatments permitted the relationship of consumption to the %ADG to be investigated (Fig. 5). The relationship between consumption and %ADG is expressed by the equation:

$$Y = -0.0015X + 0.1451X^2 - 0.4459 \quad (p < 0.05)$$

where X = consumption in mg per g fish per day and Y = % daily gain.

From this relationship the following parameters were deduced: (a) the maintenance ration (the point of intersection of the X axis by the curve) was 3.18 mg; (b) the optimal ration (point of intersection of the tangent with the curve) was 16 mg resulting in a %ADG of 1.52; and (c) the ration at which the maximum performance was obtained (the asymptotic point) was 42 mg.

The FCR and PER ranged from 1.52 to 3.79 and 0.86 to 2.14. The best FCR (1.52) and PER (2.14) was observed in the smallest size group of *O. niloticus* fed *ad libitum*/day, whereas the poorest were observed in O. niloticus young of size Gr. III fed *ad libitum*/day. Within a size group between treatments however, the FCR or the PER differed significantly from each other only in a few instances.

Generally, the efficiency of food and protein conversion into fish flesh decreased with increasing size. Also, The FCR and PER were more favorable in fish maintained on a predetermined ration fed once or twice daily compared to those fed thrice or four times a day. However, the above trends were not that clear cut when fish were fed *ad libitum* (Fig. 6).

Discussion

Changes in daily food consumption have been reported for most species and variations have been correlated to size and/or age (Pandian 1970; Birkett 1972; De Silva and Perera 1983), salinity (Kinne 1960; De Silva and Perera 1976; Dendrinos and Thorpe 1985), and temperature (Boehlert and Yoklavich 1983) among other factors. Grayton and Beamish (1977) also observed that the intake of rainbow trout was higher during the initial week or two of the experimental period, as in the present study. The pattern of this variability in food consumption has been investigated only superficially (De Silva and Perera 1983) and, as pointed out by these authors, it could be even related to daily changes in protein metabolism and the like also resulting in daily variability of the digestibility of the ingested material. Charles et al. (1984) observed that the feeding frequency increased consumption in *Cyprinus carpio* fry up to a point and thereafter further increases in frequency did not result either in an increase or decrease of intake. The findings of the present study also indicate that feeding frequency influences intake but the trends are not that clear cut.

Feeding frequency is known to affect fish growth (Kono and Nose 1971; Grayton and Beamish 1977; Brett 1979) and food conversion efficiency (Andrews and Page 1975; Greenland and Gill 1979; Charles et al. 1984). It is evident from the present study that the performance in terms of per cent average daily weight gain (%ADG), food conversion ratio (FCR) and protein efficiency ratio (PER), all important and reliable indicators of performance, was not only affected by the feeding frequency but also by the ration size. For example, in *O. niloticus* of body weight

6.84-8.08 g the % gain in weight increased with increasing feeding frequency when fed *ad libitum* each time. However, when a ration of 3% body weight was dispensed at different frequencies the %ADG was maximal in those fish receiving the ration once a day in the morning. Furthermore, the %ADG in the latter case was better and the FCR and PER in the two instances did not differ from each other significantly ($p > 0.05$). These observations point to the fact that within the same species, fish of varying sizes could perhaps react differently.

Restricted feeding is known to improve the growth performance in mammals (Leville 1970). Jobling (1983) has pointed out that feeding fish to satiation at a particular feeding frequency can minimize food wastage compared to *ad libitum* feeding, provided that full feeding opportunity is available to every individual, thereby preventing any growth depensation due to social interactions (Jobling and Wandsvik 1983). In these experiments however, obvious effects of growth depensation where individuals lost weight were not evident. In addition, feeding time is also known to affect performance in certain species of fish (Noeske et al. 1981; Sundararaj et al. 1982). There is increasing evidence that most fish species have a preferred feeding time and according to Noeske and Spieler (1984) the optimal feeding rate is likely to differ depending on feeding time. The present experiments were not designed to test the optimal feeding time for *O. niloticus* young. Also to the authors' knowledge, experimental studies on cichlids to test the optimal feeding time have not been carried out. It was also evident that the effect of feeding frequency on conversion was less marked in *O. niloticus* fed a predetermined ration unlike in the case of *Cyprinus carpio* (Charles et al. 1984; Goolish and Adelman 1984).

The present experiments also provide evidence that proper husbandry practices may help save on feed (also see Jobling 1983; Andrews and Page 1975). It is clear that in *O. niloticus* young *ad libitum* feeding could be wasteful, that comparable or better results can be obtained by feeding restricted rations at proper frequencies and that different size groups of *O. niloticus* might respond differently to different feeding frequencies.

Acknowledgements

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Table 1. Composition of the test diet.

Ingredient	% (dry wt.)
Fishmeal	40.0
Soyabean	16.0
Coconut meal	13.0
Wheat flour	19.0
Fish oil (cod liver oil)	3.0
Plant oil (soya oil)	6.0
Vitamin mixture	0.265
Mineral mixture	1.0
Cr_2O_3	1.0
Kaopex	0.735
Proximate composition	
Protein	30.78
Lipid	18.02
Ash	16.45
Cal. value Kcal g^{-1}	4.6925

Table 2. Nature of husbandry aspect and details (± S.D.).

	Size group			
	I	II	III	IV
Weight (g)				
Mean ranges	0.8760 – 1.0810	3.0374 – 3.3609	6.8464 – 8.0812	14.41 – 17.80
Individual wt. ranges	0.5415 – 1.7713 ±0.2971	3.8527 – 5.0217 ±0.91	5.1038 – 8.9246 ± 1.6079	
No. of fish per tank	20	15	10	08
Feeding strategy				
Frequency	1/D, 2/D, 3/D, 4/D	2/D	1/Dm, 1/Da, 3/D, 4/D, 2/D	4/D
Ration	*Ad libitum*	2%, 3%, 4%, 5%, 6% b.wt. day^{-1} and *ad libitum*	3% body wt. day^{-1} and *ad libitum* (1/D; 2/D; 4/D)	*Ad libitum*

1/Dm, 1/Da = once each day in morning and afternoon, respectively.

Table 3. Mean overall daily food consumption body weight/day with the S.D. for groups of *O. niloticus* fed *ad libitum*, range in weekly consumption means in parentheses. Means with the same superscript along each vertical line are not significantly different from each other at the 5% level.

	Size group			
Frequency		II	III	IV
1/day	3.066^{a} ± 0.92 (2.048 – 4.436)	–	1.537^{a} ± 0.59 (1.044 – 2.271)	–
2/day	3.904^{ab} ± 0.91 (2.443 – 7.294)	2.87 ± 0.80 (1.772 – 6.038)	2.128^{ab} ± 0.65 (1.696 – 2.608)	–
3/day	4.242^{ab} ± 0.90 (2.626 – 8.166)	–	2.370^{ab} ± 0.84 (1.916 – 2.834)	–
4/day	4.78^{b} ± 0.82 (2.514 – 10.321)	–	2.866^{b} ± 0.83 (1.809 – 3.316)	2.40 ± 0.44 (1.834 – 2.868)

Table 4. Average daily gain (ADG)[1], food conversion ratio (FCR)[2] and protein efficiency ratio (PER)[3] of *Oreochromis niloticus* young in three size groups under different husbandry regimes. For any one experimental group parameters with the same superscript are not significantly different ($p < 0.05$)

Size gr. (Mean weight, g)	Ration (% body wt./day)	Frequency (times/day)	% ADG	FCR	PER
Gr. I (0.8760–1.0810)	Ad libitum	1/D	3.51^{a}	1.52^{a}	2.14^{b}
		2/D	6.0^{bc}	1.54^{a}	2.10^{b}
		3/D	4.77^{b}	1.73^{b}	1.87^{a}
		4/D	6.20^{c}	1.82^{ab}	2.00^{ab}
Gr. II (3.0374–3.3609)	2	2/D	1.52^{a}	1.96^{a}	1.96^{b}
	3	2/D	2.57^{b}	2.02^{a}	1.80^{b}
	4	2/D	3.29^{b}	2.51^{a}	1.29^{ab}
	5	2/D	3.17^{b}	3.21^{b}	1.01^{a}
	6	2/D	2.85^{b}	3.64^{b}	0.89^{a}
	Ad libitum	2/D	2.44^{b}	1.89^{a}	1.92^{b}
	Starved	–	0.41	–	–
Gr. III (6.8464–8.0812)	3	1/Dm	2.43^{b}	2.27^{a}	1.43^{a}
		1/Da	2.85^{b}	2.13^{a}	1.51^{a}
		2/D	2.51^{b}	2.10^{a}	1.54^{a}
		3/D	1.93^{a}	2.42^{a}	1.33^{a}
		4/D	2.08^{a}	2.29^{a}	1.41^{a}
	Ad libitum	1/D	0.44^{a}	3.79^{b}	0.86^{a}
		2/D	0.73^{a}	3.56^{b}	0.91^{a}
		3/D	0.99^{b}	2.58^{a}	1.26^{b}
		4/D	2.14^{c}	2.08^{a}	1.56^{b}
Gr. IV (1441–1780)	Ad libitum	4/D	1.86	1.86	1.76

[1] Expressed as percentage, [2] Food consumed/increase in weight (g), [3] Increase in weight/protein consumed (g).
1/Dm and 1/Da, fed once daily in the morning and afternoon, respectively.

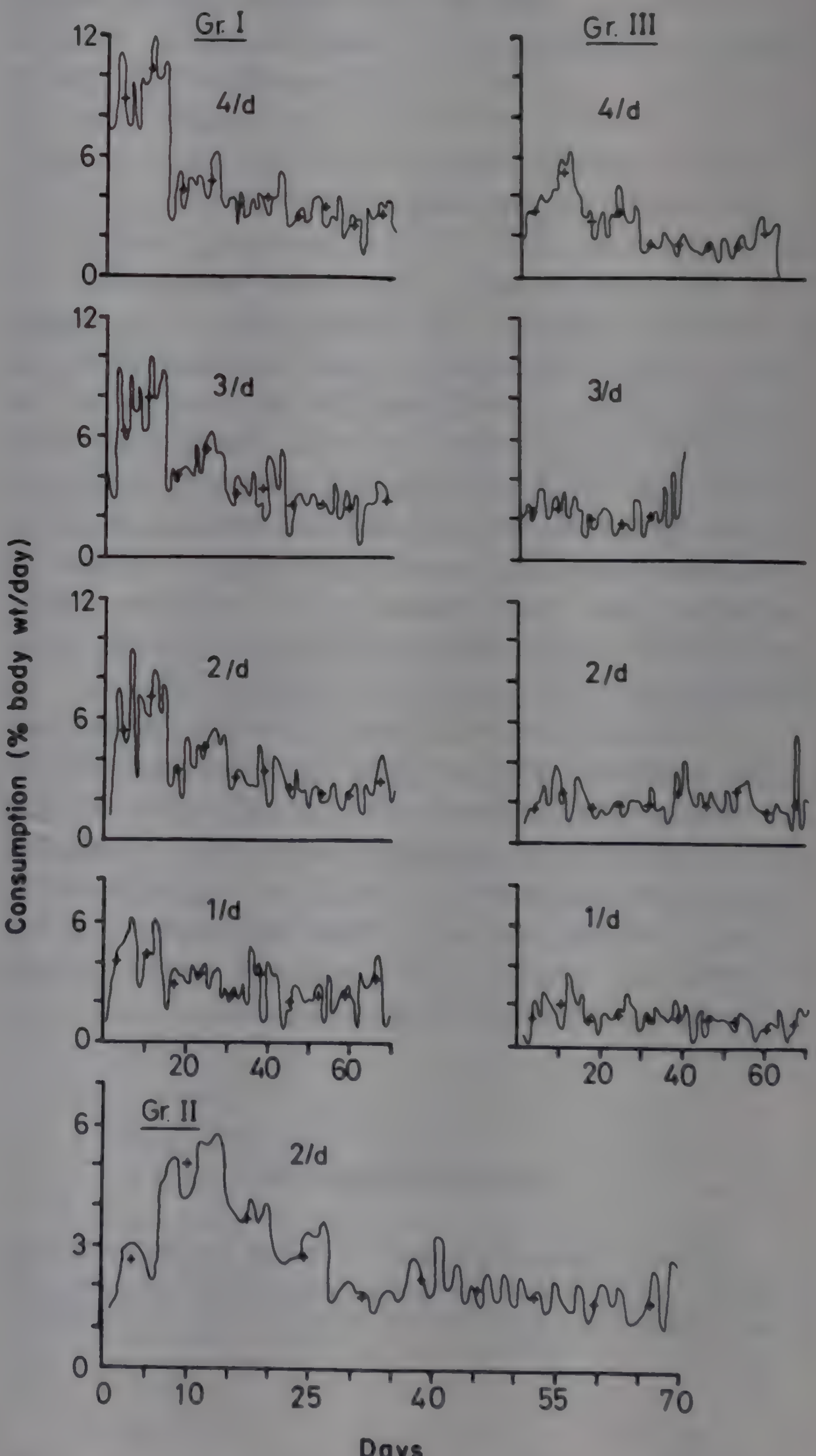

Fig. 1. Daily food consumption of size groups I-III (see Table 2) of *O. niloticus* young maintained under different dietary regimes. + denotes the mean weekly intake.

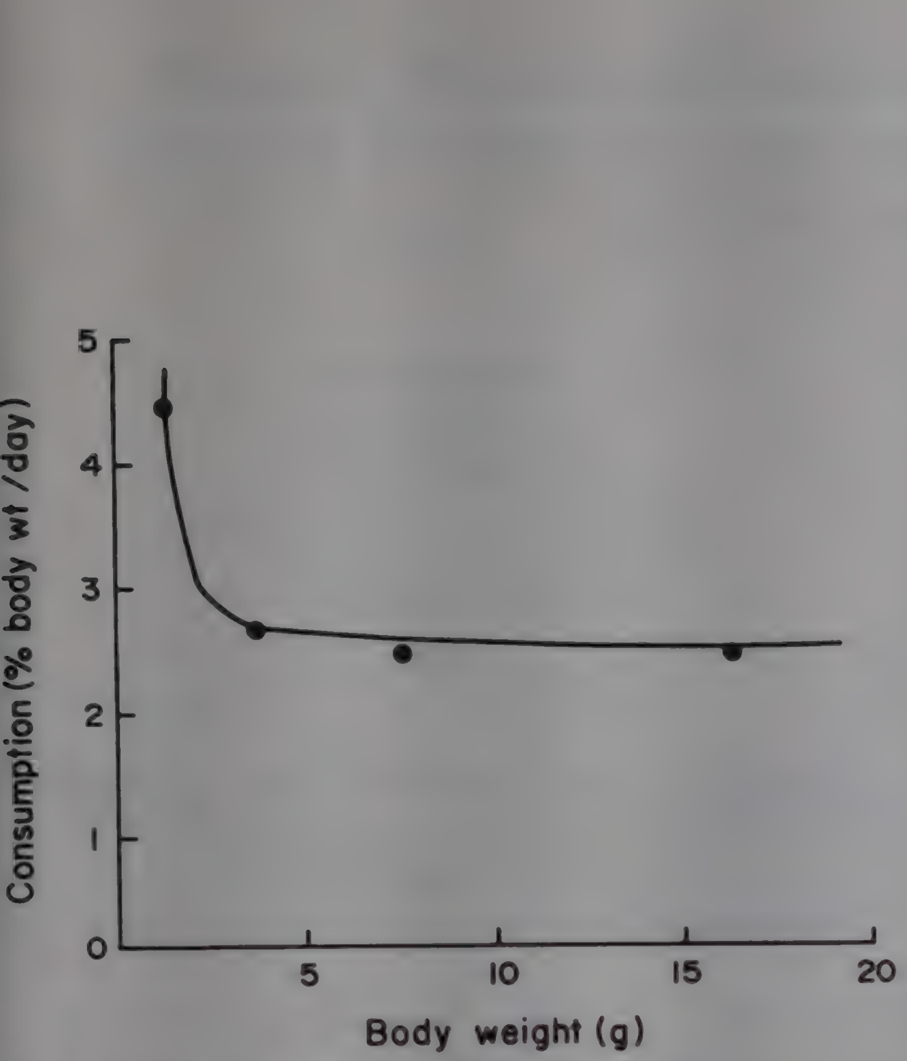

Fig. 2. Relationship of mean body weight to mean consumption in *O. niloticus*.

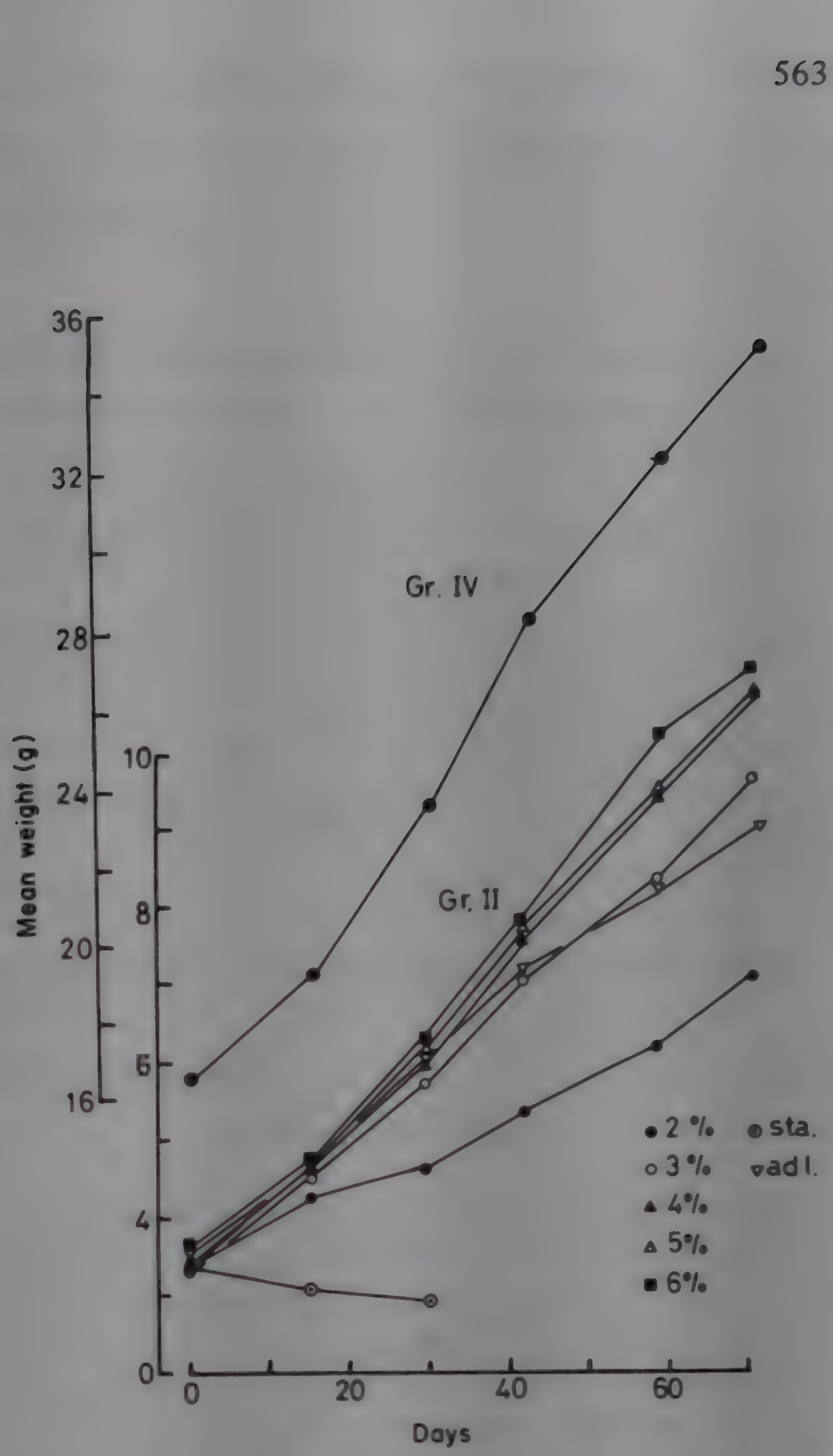

Fig. 4. Mean fortnightly weight of *O. niloticus*, Gr. II and IV (see Table 2) maintained on predetermined rations or *ad libitum*, fed twice a day.

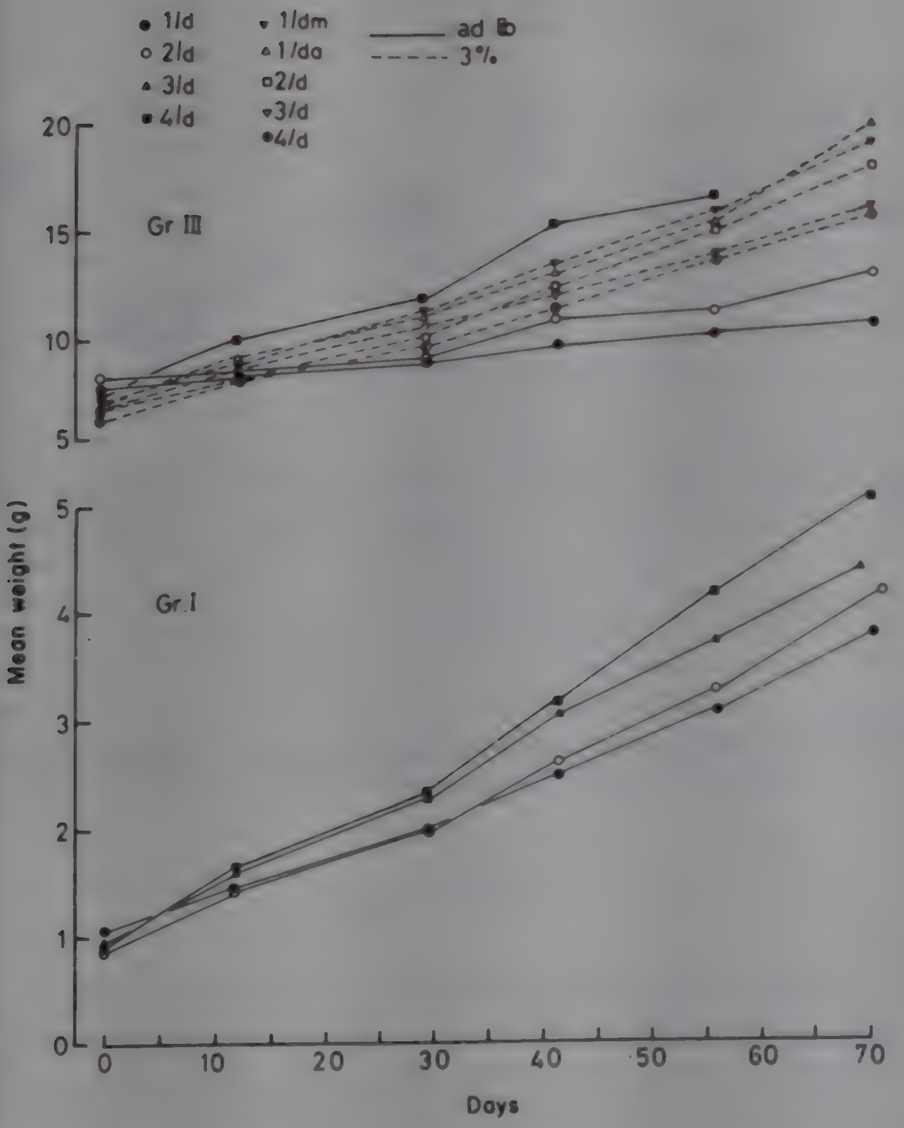

Fig. 3. Mean fortnightly weight of *O. niloticus* young, Gr. I and III (see Table 2) maintained under different feeding frequencies and fed *ad libitum* or at 3%.

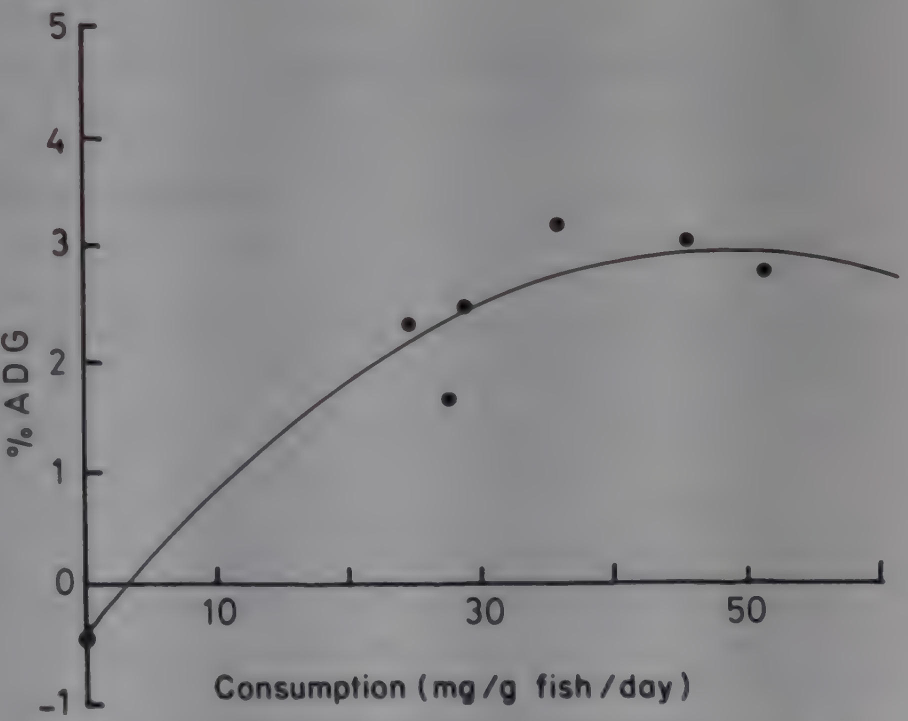

Fig. 5. Relationship between mean consumption and per cent average daily gain in weight in *O. niloticus*, Gr. II (see Table 2).

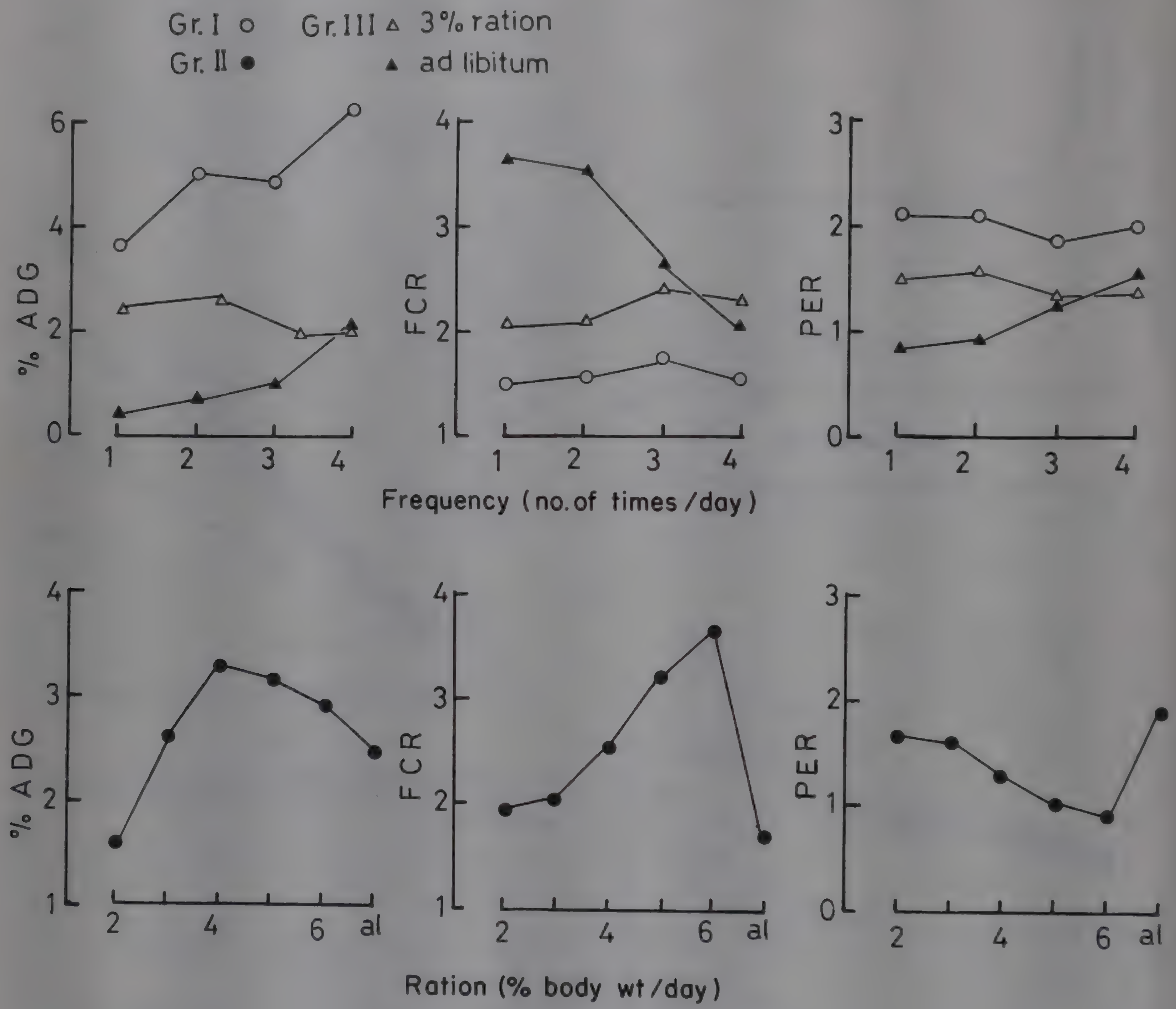

Fig. 6. Changes in % ADG, FCR and PER of *O. niloticus* young of different size groups in relation to feeding frequency and ration size.

Effects of a Combination of Poultry Manure and Varying Doses of Urea on the Growth and Survival of Cultured Carps

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Abstract

Poultry manure is the best among the commonly used organic manures in India. Urea in terms of nitrogen, is more economical than other nitrogenous fertilizers. This work studied the effects of fertilization of fishponds with a combination of poultry manure and urea and determined the most economical dose of urea to be used with poultry manure.

Twelve uniform-sized cement cisterns of 25 m^2 each were used for the experiment which was conducted in 133 days. Three different doses of urea (46% N) at 100 kg/ha, 300 kg/ha and 500 kg/ha, in combination with a constant quantity of poultry manure (2,000 kg/ha) were tried. All the cisterns were fertilized twice during the experiment. In the second installment, the dosage was reduced to half for both poultry manure and urea. Fish were stocked a week after the initial fertilization at 8,000/ha, using uniform-sized silver carp, catla, rohu and common carp fingerlings in the ratio of 1:1:1:1. Fish growth was assessed fortnightly. Final growth attained by the fishes was significantly different between treatments as well as between species. High concentration of ammonia (2.4-3.0 ppm) in medium urea treatment and high urea treatment resulted in mass mortality of all the fishes stocked by day 14. The cisterns were restocked with fishes of identical size on the same day. The overall survival was highest in medium urea treatment. Total net fish production was highest in low urea treatment because of better growth of silver and common carp. It appears that poultry manure and low dose of urea give the best results for carps. Therefore 100 kg/ha of urea, with 2,000 kg/ha of poultry manure is the safe and economical dose for carp culture.

Introduction

Fertilization is the cheapest and simplest means of increasing aquatic productivity. Poultry manure is the best among the commonly-used fertilizers and is popularly used in China, Philippines, India, Israel and other countries. Urea is a biocomponent nitrogenous fertilizer containing approximately 46% pure nitrogen. It is a physiologically neutral fertilizer as aquatic plants assimilate both ammonia and carbon dioxide released out of urea by urease hydrolysis (Wolny 1967). Urea is reported to be the most economical nitrogenous fertilizer per kg of nitrogen. A combination of organic and inorganic fertilizers has been reported to be better than the application of only one of them (De Bont 1965; Rabanal 1967; Shankar and Varghese 1981). Hence, this study of the effect of pond fertilization with a combination of poultry manure and urea. However, urea being a costly fertilizer, it was necessary to determine its most economical dose to be used with poultry manure. Therefore, the effect of different doses of urea in combination with a constant dose of poultry manure was studied.

Materials and Methods

Twelve cement cisterns of uniform size (5 x 5 x 1 m), 25 m^3 in area, each provided with soil bed of about 15 cm thick, were used for the experiment which lasted 133 days. The cisterns were drained and limed with quicklime at 400 kg/ha and dried for about a week. Later, water from an adjacent perennial well was pumped into all the cisterns to about 70 cm.

Nine cisterns were fertilized with a combination of poultry manure and urea (46% N), while the remaining three only with poultry manure to serve as controls and were designated as PM treatment. All the twelve cisterns were fertilized twice during the experiment. In the first application poultry manure was applied at a rate of 2,000 kg/ha dry weight in all the cisterns. In addition to poultry manure, three doses of urea were applied in nine cisterns, a set of three cisterns receiving one dose. The dosages of urea added were 100, 300 and 500 kg/ha and were designated as low (PL urea), medium (PM urea) and high (PH urea), respectively. In the second application, applied 82 days after the first, dosage was reduced to half that of the first for both poultry manure and urea.

Each cistern was stocked with five fingerlings each of silver carp, *Hypophthalmichthys molitrix* (Valenciennes); catla, *Catla catla* (Hamilton); rohu, *Labeo rohita* (Hamilton) and common carp, *Cyprinus carpio* var. *communis* (Linnaeus), a week after the initial fertilization. The total stocking density reached 8,000 fish/ha.

Water and plankton samples were collected from each cistern weekly. Water samples were analyzed for pH, free CO_2, dissolved oxygen, alkalinity, dissolved organic matter, ammonia, nitrate-nitrogen and phosphate-phosphorus. Plankton samples were analyzed quantitatively and qualitatively. Soil samples were collected monthly and analyzed for various parameters.

Fish growth was assessed fortnightly by collecting a minimum of 50% of the stock to record the individual length and total weight of each species. At the end of the experiment, the cisterns were drained and the individual length and the total weight of each species from each cistern recorded. Air and water temperatures were noted at each sampling day. Two-way analysis of variance was applied to find out the significance of difference in fish growth and survival among treatments.

Results

The average final weight attained by each species differed significantly among treatments. But there was no significant difference in growth of each species among cisterns under the same treatment; the data from the triplicate cisterns were pooled and the average values calculated. Average weight increased in all treatments. Growth of silver carp was remarkably better with PL urea than in the others (Fig. 1). Growth of catla was highest with PM followed by PH urea, PM urea and PL urea in that order (Fig. 2). Rohu attained the highest growth rate with PH urea and the lowest with PM urea. This species attained average weights of 114.0, 90.6, 130.44 and 105.43 g with PL urea, PM urea, PH urea and PM, respectively. Average weight of common carp incresed from 4.0 g in all treatments to 181.0, 181.46, 145.0 and 162.0 g in PL urea, PM urea, PH urea and PM treatments, respectively.

During the first fortnight of the rearing period, in one of the cisterns under PM urea treatment and in all the three cisterns of PH urea treatment, mass mortality of all the stocked fishes was observed. These four cisterns were restocked with fishes of identical size on the 14th day after initial fertilization. In these cisterns restocking day was taken as the day of stocking and the experiment was extended for 14 days to maintain a uniform rearing period for all the cisterns.

Percentage survival of different species in the various treatments is given in Table 1. There is significant difference in total survival among treatments, but no significant difference among species at 10% level of significance.

Average net production of silver carp was highest in PL urea (Table 2), while the net production of catla was the highest with PH urea. Total average net production was highest with PL urea and lowest with PH urea. The cost of producing 1 kg of fish, taking into account only the cost of fertilizers, was Rs.1.46 for PM, Rs.1.59 for PL urea, Rs.2.41 for PM urea and Rs.4.30 for PH urea treatments.

Discussion

Silver carp is phytoplanktophagic in its food habit (Hora and Pillay 1962). Average phytoplankton production was highest in PL urea treatment and this obviously was the reason for the remarkable growth of silver carp in this treatment. Phytoplankton, in terms of number per liter, was more abundant in combination treatments. Silver carp grew better in combination treatments, except with PH urea. The average ammonia content was the highest (0.3864 ppm) with PH urea which could have adversely affected the growth of silver carp in this treatment.

The growth trend of catla in the different treatments was observed to be the reverse of that of silver carp. Average dry weight value of total plankton (35.16 mg/100 ml), and the the number of zooplankton was the highest with PH urea. Thus, the quantity of total plankton and the high number of zooplankton available appear to have influenced the growth of catla. The high concentration of ammonia with PH urea did not appear to have adversely affected the growth of catla. Zooplankton production with PM was fairly good, which could have influenced the growth of catla in that treatment. Natarajan (1976) recorded fastest growth of catla in poultry manure treated cisterns compared to cattle dung and sewage sludge treatments. The above results indicate that poultry manure alone is sufficient for good growth of catla.

The difference in growth of rohu among the treatments was not marked. Harish (1979) reported better growth of rohu in a combination of organic manure and NPK as compared to NPK alone. The poor growth of common carp with PH urea could be due to the high ammonia content (Reid and Wood 1976).

The mass mortality of all the fishes in one of the cisterns with PM urea and in all the three cisterns with PH urea during the first fortnight was due to the high concentrations of ammonia (ranging from 2.4 to 3.0 ppm) in those cisterns. Reid and Wood (1976) stated that free ammonia in concentrations over 2.5 mg/l in neutral and alkaline waters is apt to be harmful to many freshwater species. During this study, water was alkaline in reaction with the pH range of 8.0 to 9.6.

The low average survival of silver carp with PH urea was due to total mortality in one of the cisterns and very poor survival (20%) in another cistern. Total mortality in one of the cisterns with PL urea led to poor average survival in this treatment also. When mortality occurred, thick blooms of *Anabaena* sp. appeared in the cistern with PH urea and those of *Microcystis* sp. and *Anabaena* sp. in the cistern with PL urea. The thick phytoplankton blooms leading to the reduction of dissolved oxygen concentration to lethal levels in the early morning hours (Banerjea 1967), could have been the cause of the mortality. Further, the toxin released by *Microcystis* sp. could have also contributed to the mortality (Phillips et al. 1985). Similarly the thick blooms of phytoplankton, especially *Anabaena* sp., *Ankistrodesmus* sp., *Coelastrum* sp. and *Mougeotia* sp., in one of the cisterns with PH urea on the 70th day could have caused total mortality of catla. Because of low survival of catla with PM, in comparison with PH urea, the net production was low in spite of almost the same rate of growth in both. Inversely, because of the high survival of catla with PM urea, it gave greater net production than with PM treatment.

Average survival of rohu was very poor with PL urea because of the total mortality of this species in two cisterns under this treatment. The highest survival and the highest net production were recorded with PM urea. The low net production of rohu with PH urea was due to low survival in two cisterns under this treatment.

Common carp showed the best survival among the four species, except with PH urea. Total mortality of common carp in one of the cisterns with PL urea, observed on the 42nd day, could be attributed to phytoplankton bloom. The low net production of common carp with PH urea was due to poor survival. PM urea gave the highest net production. PM urea and PL urea gave almost the same and the highest net production.

On the whole, common carp contributed largely to net production with all the treatments, except with PH urea to which catla contributed the highest. The least contribution to net production in all the tratments was by rohu.

Total net fish production was the highest with PL urea because of the better growth of silver carp and common carp, followed by PM urea, PM and PH urea treatments, in that order.

The cost of production per kg of fish was highest with PH urea. The slightly higher cost of production with PL urea than with PM can probably be ignored since the total production, as well as the total profit obtainable in the former treatment, was significantly greater than with PM.

It can be concluded that poulty manure with low dose of urea gives the best results for silver carp and common carp culture. There is no distinct difference in the rate of growth of rohu due to the fertilizer effect and poultry manure alone is sufficient for good growth of catla. Higher doses of urea not only failed to increase the productivity but also resulted in high fish mortality within the first fortnight of rearing due to excessive release of ammonia from urea. Occurrence of this heavy mortality suggests that urea should be used with caution as a fishpond fertilizer. Being a costly fertilizer, excessive use of urea will also adversely affect the economics of fish culture. Therefore, 100 kg/ha of urea in combination with 2,000 kg/ha of poultry manure is the safe and economical dose for carp culture.

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Table 1. Percentage survival of different species in the various treatments.

Treatment	Cistern No.	Species Silver carp	Catla	Rohu	Common carp
PL Urea	1	nil	20	nil	nil
	2	80	60	nil	100
	3	60	80	60	100
	Average survival	46.66	53.33	20.00	66.66
PM Urea	1	80	100	100	100
	2	40	80	40	100
	3	60	60	100	100
	Average survival	60.00	80.00	80.00	100.00
PH Urea	1	nil	nil	20	20
	2	20	100	20	40
	3	60	100	60	60
	Average survival	26.66	66.66	33.33	40.00
PM	1	20	60	100	100
	2	80	80	60	100
	3	60	20	40	100
	Average survival	53.33	53.33	66.66	100.00

Table 2. Average fish production in different treatments.

Treatment	Species	Average net production in 126 days g/cistern	kg/ha	Average net fish production kg/ha/yr	Total average fish production kg/ha/yr
PL Urea	Silver carp	842.65	337.06	976.40	
	Catla	334.00	133.60	387.01	
	Rohu	332.00	132.80	384.70	
	Common carp	885.00	354.00	1,025.48	2,773.59
PM Urea	Silver carp	565.33	226.13	655.06	
	Catla	542.00	216.80	628.03	
	Rohu	342.00	136.80	396.28	
	Common carp	887.33	354.93	1,028.17	2,707.54
PH Urea	Silver carp	250.99	100.40	290.84	
	Catla	989.65	395.86	1,146.74	
	Rohu	201.33	80.53	233.28	
	Common carp	293.00	117.20	339.51	2,010.37
PM	Silver carp	350.33	140.13	405.93	
	Catla	499.99	199.99	579.34	
	Rohu	337.80	135.12	391.42	
	Common carp	790.00	316.00	915.40	2,292.09

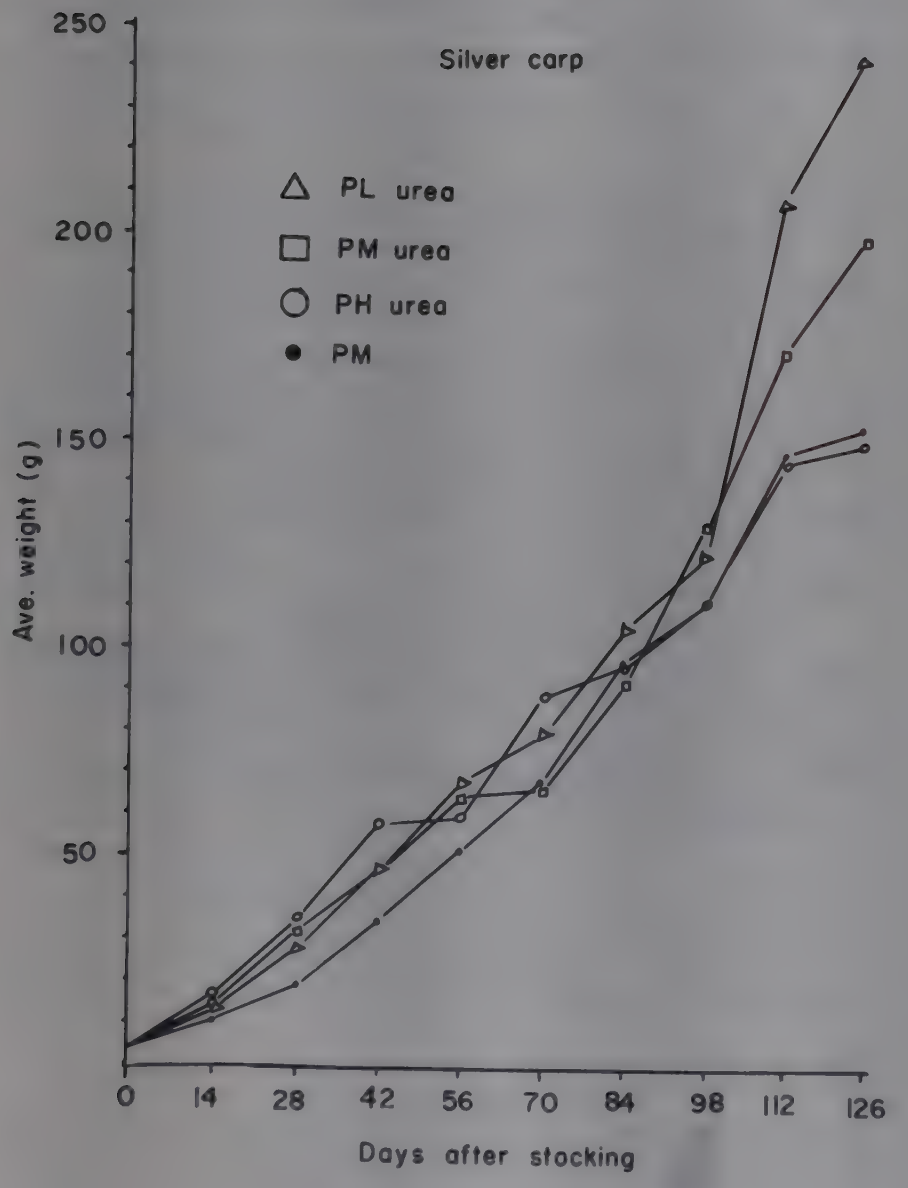

Fig. 1. Average weight (g) attained by silver carp in different treatments.

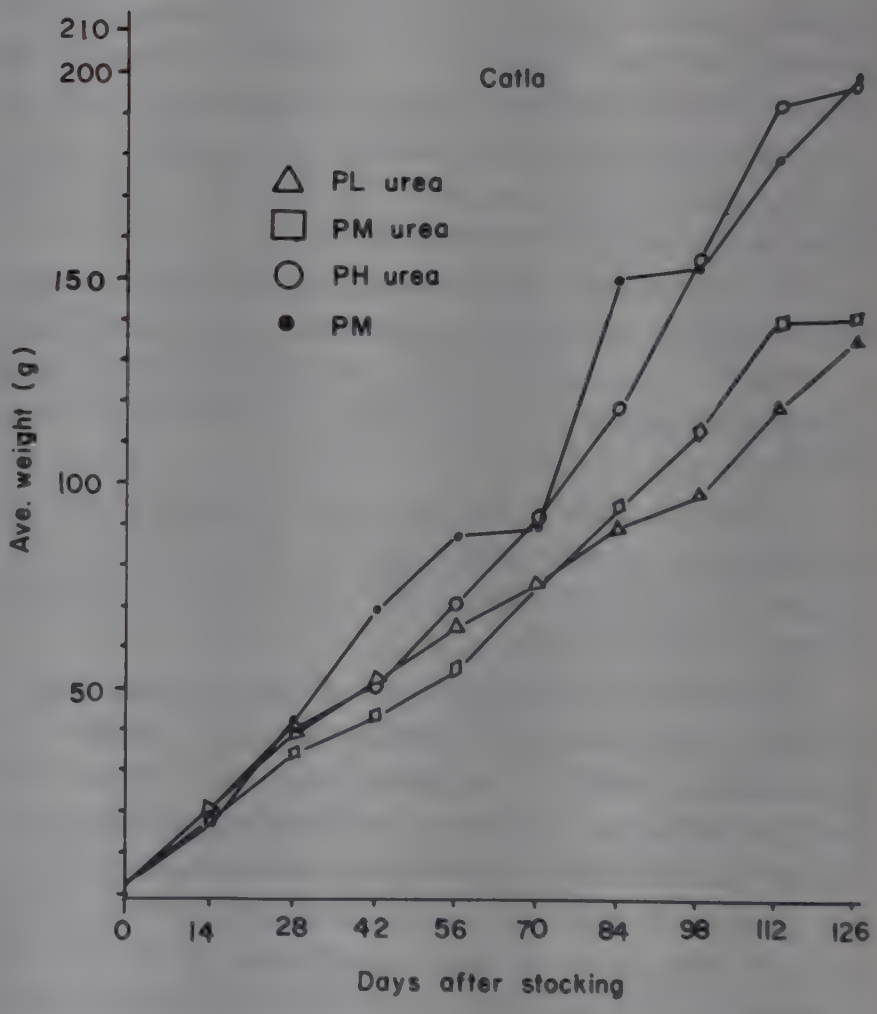

Fig. 2. Average weight (g) attained by catla in different treatments.

Diet and Feeding Chronology of Mesopelagic Micronektonic Fish, *Diaphus suborbitalis,* in Suruga Bay, Japan

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Abstract

Diaphus suborbitalis has feeding periodicity, with more intensive feeding from midnight to dawn than at dusk or early evening, and without intensive feeding at daytime. Intensive feeding time differs with fish-size; a high percentage of stomach fullness appears before sunrise in smaller fish, at midnight in larger fish and twice (one at midnight and one before sunrise) in medium-sized fish. This phenomenon in medium-sized fish appears to be an efficient strategy before the summer breeding season in terms of energetics.

Composition of stomach contents differed somewhat from season to season. Copepods composed the most diverse category, constituting 97% of the prey. Medium-sized fish fed selectively on euphausiids and *Calanus* . The food and feeding habits of *D. suborbitalis* and sergestid shrimp reveal that these two organisms do not have a prey-predator relationship; rather, they are competitors for food.

Introduction

Research on mesopelagic micronektonic fish has recently increased because of their role in biological production and food chains and great potential as fishery resources, thus necessitating the development of collecting methods.

Diaphus suborbitalis is the most important organism of about 35 species of Myctophidae found in Suruga Bay, Japan, and plays an important role in biological production. This paper describes the food, feeding habits and the ecological niche of *D. suborbitalis* in Suruga Bay.

Materials and Methods

Samples were collected mainly in Suruga Bay by R/V *Tansei Maru* of the Ocean Research Institute, University of Tokyo, and a commercial shrimp fisheries vessel from May 1973 to June 1977. Samples were taken with a 1.8-m Isaacs-Kidd midwater trawl (IKMT, 5 mm x 5 mm mesh), a shrimp midwater trawl, 163-m long by 75-m wide (13 mm x 13 mm mesh at cod end) and a Motoda plankton net with a mouth diameter of 56 cm (MTD, 0.33 mm x 0.33 mm mesh).

The stomachs of 498 fish were dissected from formalin-preserved specimens. The amount of food (state of fullness) was assessed visually: state 0, empty; state 1, partly full (< 1/2 full); state 2, relatively full (1/2 full<); state 3, very full. To investigate the feeding rhythm by time a day was divided into eight time zones; dusk, early night, midnight, late night, dawn, early part of the day, middle part of the day and late part of the day. The Ivlev index E was calculated as a measure of selectivity in which

$$E = (ri - pi) / (ri + pi),$$

where ri is the portion of the food items identified in the stomach, and pi is the portion of the food items caught in MTD net.

Results

Fig. 1 shows the diel variation of stomach fullness by collecting time. Although the seasonal variation was not considered in Fig. 1, the results by season were similar to that in Fig. 1. These data indicate that the species has a feeding cycle, with more intensive feeding from midnight to dawn than at dusk or a little later.

For convenience, fish length was divided into small fish (15-30 mm SL), medium-sized fish (30-50 mm) and large fish (> 50 mm), and the relationship between the fish size and the diel feeding activity was dealt with (Table 1). Medium-sized fish have two peaks of feeding activity, one at midnight and one at dawn, but large fish have only one peak. Consequently, with the passage of time (Fig. 1) the two peaks are mainly influenced by medium-sized fish.

To complement the insufficient day-materials in this bay, day-materials from neighboring Sagami Bay were used. Seven specimens were caught: 2 were in state 1 of unidentifiable stomach contents, 3 in state 0 condition, and 2 collected at dusk, in state 1 of identifiable stomach contents. The findings suggest that this species has no intensive feeding at daytime. The depths of main feeding activity can be presumed by the appearance of state 1, by collecting layer and by the variation of stomach fullness. Considering the result of diel migration (Go 1980), the feeding activity is vigorous when this species surfaces above the 100-m layer at night, but is feeble at the 200-m layer at daytime. The feeding rate was calculated from the stomach contents after two hours tow netting, assuming that all the stomachs of the fish were empty when tow netting began. The number of food organisms and their individual weight tended to increase with fish size. The average feeding rate of medium-sized fish was 0.65 individuals/min. and 0.16 mg/min. Considering that stomach fullness is low in early night real values may be underestimated from the average feeding rate.

The frequency of food organisms in the stomach is shown in Table 2. The number of identified food organisms was 8,739, of which 99.1% were Crustacea. Of the Crustacea, copepods made up 97%.

Food organisms varied somewhat by fish size, *Oncaea* and *Paracalanus* occurring frequently in small fish, *Paracalanus* and *Calanus* in medium-sized fish and *Calanus* in large fish. It appears, despite the small sample, that the feeding habits of large fish seem to change from copepods to large organisms (e.g., euphausiids).

Generic composition of stomach contents differed somewhat from season to season. In winter *Oncaea* and *Paracalanus* were mostly consumed as food, in spring *Calanus* and *Paracalanus*, in autumn *Euchaeta* and euphausiids. Such a seasonal change of food organism is consistent with the variation of food organisms according to fish size: small fish in winter, medium-sized fish in spring, and large fish from summer to autumn.

The gill raker interval was 0.20-0.35 mm in small fish, 0.30-0.50 mm in medium-sized fish and 0.50-0.75 mm in large fish. Small fish with 0.23-mm gill raker interval on the average fed mainly on less than 1-mm sized food, medium-sized fish with 0.40-mm interval on 1-mm sized food and large fish with 0.63-mm interval on 2-mm sized food.

The relationship between the fish size and the largest size of food organisms was

$$Pm = 0.02 \times l^{1.78} \ (r = 0.98)$$

where Pm is the largest food size (mm), l is the fish size (mm), and r is the coefficient of correlation. The largest food organism eaten by this species was a sergestid shrimp of 37 mm (0.18 g), corresponding to more than half the size of the 67-mm predator and to 5% of its weight. The relationship between the food weight for satiation (state 3) and the fish weight was:

$$Sw = -7.66 + 0.04\ Fw \ (r = 0.84)$$

where Sw is the wet weight (mg) of food, and Fw is the wet weight (mg) of fish.

Specimens collected simultaneously by IKMT and MTD (22nd, 2201--23rd, 0005, April 1977) were used to ascertain food selectivity (Table 3). Medium-sized fish had high food selectivity for euphausiids (E = 0.60), and low food selectivity for *Calanus* (E = 0.21). An analysis by simultaneous towing of IKMT and MTD nets was attempted, but only one towing time was used. Thus, few data are available on food selectivity of small and large fish.

Discussion

Hopkins and Baird (1977) gave a comprehensive review of the feeding of mesopelagic fish. This review and the results of this study show that migrants which ascend above 200 m at night have a feeding cycle, while small-scale migrants or non-migrants appear to have no feeding cycle.

There are instances of variations of feeding cycle according to ontogeny even in the same species (Gorelova 1975). This phenomenon was also displayed in this research. This feeding phenomenon in the medium-sized fish appears to be an efficient strategy before the breeding season, summer, in terms of energetics (Go 1981). According to recent research, food organisms vary with ontogeny. Nonetheless, Crustacea like copepods, euphausiids, amphipods and small shrimp are composed of important food organisms.

Little work has been done on the seasonal variation of food organisms of mesopelagic micronektonic fish (Cailliet 1972; Gj saeter 1973). Such seasonal variation of feeding ecology depends on fish species and size. Its important cause may be the standing stock of zooplankton and the seasonal variation of zooplankton biomass.

D. suborbitalis tends to feed on larger food organisms with increasing fish size. Evidence of this feeding pattern appeared in *Maurolicus muelleri* (Okiyama 1971), *Sternoptyx diaphana* (Hopkins and Baird 1973) and myctophids in the tropical western Pacific (Gorelova 1975). It is postulated that myctophids are filter-feeders and food size is controlled by the gill raker interval used in filtering (Okiyama 1977). For fish which live all day below 200 m, where zooplankton biomass is small, the size of food organisms is not important. Food ranging from small to even bigger than the fish is taken. It suggests that such fish are well adapted to poor environment, taking any food organism regardless of food size.

As a filter-feeder, myctophids tend to ingest food organisms of a wide size range depending on their gill raker interval (Okiyama 1977), indicating food selectivity according to size. Apart from the present paper, Merrett and Roe (1974) reported food selection from the results of simultaneously-collected materials.

There have been a few reports on prey-predator relationships in Suruga Bay's biological community (Kubota 1973; Hogetsu and Taga 1977). The diagram of food relationship centering around *D. suborbitalis* obtained from the above findings and the present research is shown in Fig. 2. This figure indicates that this species is in the middle position which connects the primary or secondary consumer, zooplankton, and the high-level consumer, nekton, in food chains. Although this species and *D. watasei* have been known as the major consumers of sergestid shrimp, an important fisheries resource in Suruga Bay (Omori 1969), only one species of shrimp was identified in the present investigation. This shrimp lives on *Calanus, Paracalanus, Clausocalanus* and *Euchaeta* in spring and *Calanus, Euchaeta, Candacia, Oncaea* and euphausiids in summer and autumn and also feeds actively from sunset to sunrise (Omori 1969). Comparison of food and feeding habits of these two species indicates that *D. suborbitalis* is not so much a consumer of sergestid shrimp but is rather its food competitor.

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Table 1. Stomach contents in the four different categories of fullness (State 0-3) in each size group at different times of the day.

	Small fish (15-30 mm)					Medium fish (30-50 mm)					Large fish (50-70 mm)				
Time	No. of	State				No. of	State				No. of	State			
range	fish	0	1	2	3	fish	0	1	2	3	fish	0	1	2	3
Du		%	%	%	%	41	12%	88%	%	%	22	9%	64%	18%	9%
En	13		69	15	15	129	11	71	14	4	86	7	73	8	12
Mn	5		60	20	20	88	2	9	33	57	19	5	16	32	47
Ln	2			50	50	58	2	29	36	33	26	8	42	35	15
De						10		30		70	3		33	67	
Ed						1		100			6		67	17	17

Du = dusk; En = early night, Mn = midnight; Ln = late night; De = dawn, Ed = early day.

Table 2. Food organisms in stomachs of 406 specimens, *Diaphus suborbitalis* collected in Suruga Bay, May 1973-June 1977.

Food items	No. of fish with food organisms	No. of food organisms in stomachs
Copepoda		
Calanus	158	3,662
Paracalanus	167	3,473
Acartia	96	307
Acrocalanus	34	166
Clausocalanus	52	133
Pleuromamma	78	116
Temora	29	84
Euchaeta	40	66
Candacia	48	51
Euchirella	28	28
Rhincalanus	3	8
Eucalanus	4	6
Lucicutia	4	5
Centropages	2	4
Labidocera	4	4
Mecynocera	1	3
Euaugaptilus	2	3
Heterorhabdus	3	3
Aetideopsis	2	2
Undinula	2	2
Aetideus	1	1
Chirundina	1	1
Undeuchaeta	1	1
Xanthocalanus	1	1
Oncaea	90	197
Corycaeus	71	144
Oithona	8	13
Sapphirina	1	1
Saphirella	1	1
Crytemnestra	2	3
Euterpina	1	1
Macrosetella	1	1
Malacostraca		
Amphipoda	6	6
Decapoda	4	15
Mysidacea	6	6
Euphausiacea	84	122
Branchipoda		
Cladocera	4	4
Ostracoda	10	15
Sagittidae	32	37
Sacrodina	3	4
Chromonadae	2	2
Ciliata	1	1
Polychaeta	2	2
Diatoms	10	17
Cyanophyta	5	3
Fish	2	2
Scale	12	22

Table 3. Food selectivity index of medium-sized fish.

Items	ri (%) (N = 4,114)	pi (%)	E
Calanus	55.1	35.6	0.21
Paracalanus	32.4	36.7	−0.06
Acartia	1.5	1.1	0.15
Acrocalanus	2.6	2.4	0.04
Clausocalanus	1.2	4.3	−0.56
Pleuromamma	0.4	2.6	−0.73
Temora	0.5	0.4	0.10
Oncaea	0.5	1.2	−0.41
Corycaeus	0.9	6.3	−0.75
Euphausiacea	0.8	0.2	0.60

ri = portion of food items in stomachs; pi = portion of food items caught in MTD net.

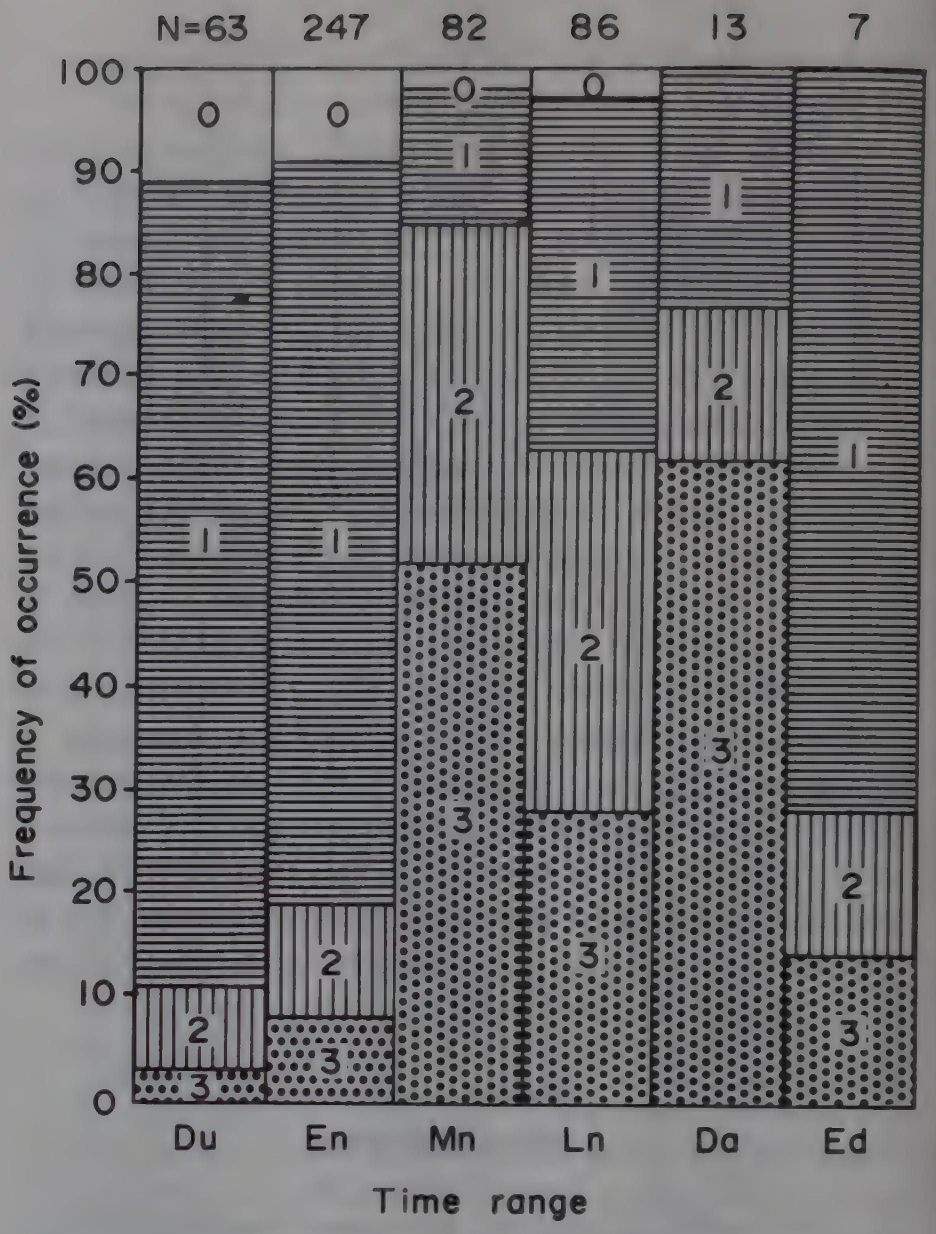

Fig. 1. Stomach composition in the four different categories of fullness (State 0-3) over the diel period for 498 specimens.

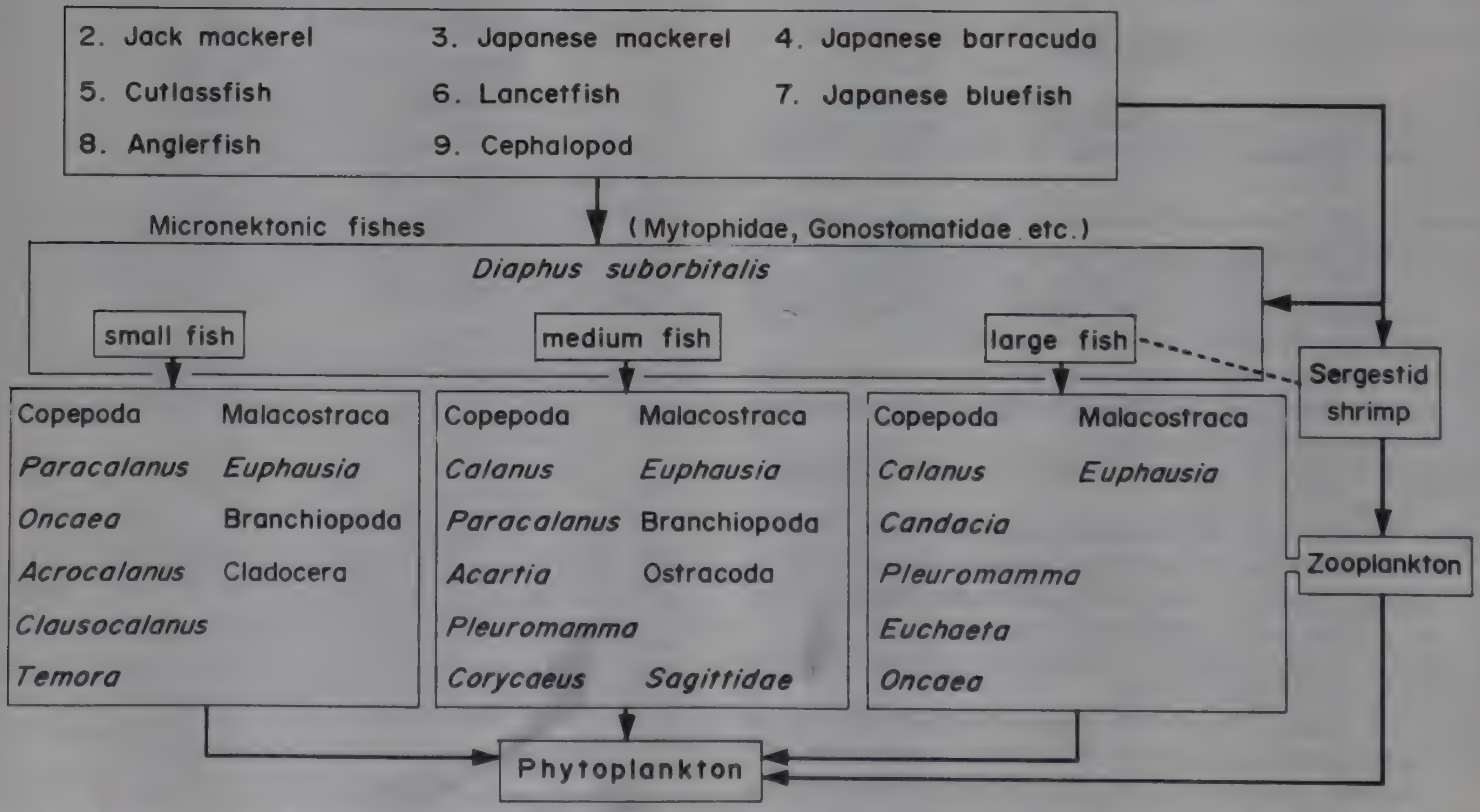
2. Jack mackerel
3. Japanese mackerel
4. Japanese barracuda
5. Cutlassfish
6. Lancetfish
7. Japanese bluefish
8. Anglerfish
9. Cephalopod
Micronektonic fishes
(Mytophidae, Gonostomatidae etc.)
Diaphus suborbitalis
small fish
medium fish
large fish
Sergestid shrimp
Copepoda
Malacostraca
Paracalanus
Euphausia
Oncaea
Branchiopoda
Acrocalanus
Cladocera
Clausocalanus
Temora
Copepoda
Malacostraca
Calanus
Euphausia
Paracalanus
Branchiopoda
Acartia
Ostracoda
Pleuromamma
Corycaeus
Sagittidae
Copepoda
Malacostraca
Calanus
Euphausia
Candacia
Pleuromamma
Euchaeta
Oncaea
Zooplankton
Phytoplankton

Fig. 2. Food web in Suruga Bay in relation to *Diaphus suborbitalis*. Present data modified after Kubota (1973) and Hogetsu and Taga (1977): (1) Sergestid shrimp, *Sergestes lucens* (2) Jack mackerel, *Trachurus japonicus* (3) Japanese mackerel, *Scomber japonicus* (4) Japanese barracuda, *Sphyraena japonica* (5) Cutlassfish, *Trichiurus lepturus* (6) Lancetfish, *Alepisaurus ferox* (7) Japanese bluefish, *Scombrops boops* (8) Anglerfish, *Crytopsaras cousesi* and (9) Cephalopod.

Feeding Habits of Larval Rabbitfish, *Siganus guttatus* in the Laboratory*

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Abstract

The feeding habits of *Siganus guttatus* larvae were determined in laboratory rearing studies at 23.8-30.3°C by examination of digestive tract contents of larvae given rotifers and/or brine shrimp. Larvae were initially fed on rotifers at a total length (TL) of 2.6 mm (day 2 from hatching), and on brine shrimp at 4.4 mm TL (day 12). A change in feeding habits, seen as the flexion point in the relationship between larval TL and maximum amount of prey, occurred at about 7.0-9.5 mm TL with rotifers as prey, and at 7.2 mm TL with brine shrimp. Higher preference for brine shrimp over rotifers was seen in larvae 8-9 mm TL and larger. These changes in habit coincided with the full osteological development of the feeding apparatus in larvae at 7-8 mm TL. *S. guttatus* larvae exhibited a diurnal feeding pattern at day 9 (mean 3.7 mm TL), day 15 (5.8 mm TL) and day 21 (7.9 mm TL). The percentage of larvae with food in the digestive tract decreased in the evening and became zero at 10 p.m. in all ages. Active feeding, when more than 50% of larvae had food in the digestive tract, shifted earlier in the day with larval growth. Satiation, when feeding first reached a plateau in the morning when food was available round the clock, occurred at 8-10 a.m. in larvae at all ages.

Introduction

Siganus guttatus is a highly esteemed food fish in the Philippines and other Asia/Pacific countries. It attains a large adult size, occurs in a wide variety of habitats and has been considered a promising aquaculture species (Von Westernhagen and Rosenthal 1976; Tahil 1978; Carumbana 1983). Spawning and larval rearing have been attempted (Palma 1978; Alcala and Luchavez 1980; Juario et al. 1985) and have recently attained a measure of repeatability with consistent survival rates (Hara et al., in press). Von Westernhagen (1974) studied the food preferences of *S. guttatus* juveniles, but there has been no study yet on the feeding habits of the larvae as related to seed production. In this paper, we describe prey preference, diurnal feeding patterns and changes in the feeding habits of larval *S. guttatus* under laboratory conditions.

Materials and Methods

To study feeding amounts and prey preference of *Siganus guttatus*, about 100,000 hatched larvae from broodstock in the Southeast Asian Fisheries Development Center (SEAFDEC) Aquaculture Department in Iloilo, Philippines, were stocked in a 5-t concrete tank on 28 July 1985 and reared until day 8 with rotifers as food at a density of about 10 rotifers/ml. On day 8, 2,000 larvae were transferred to each of three cylindrical plastic tanks containing 500 l of water for experiments with three feeding treatments: (1) rotifers only at density of about 10/ml; (2) artemia nauplii only at density of about 1/ml; and (3) combination of rotifers and artemia nauplii at the same respective densities as above.

In all cases, feeding was done at 9 a.m. daily. One hour after feeding, samples of 10 larvae were removed from the 5-t tank from day 2 until day 8, and from each of the three treatments thereafter until days 25-28. The tank bottom was cleaned and the water changed starting day 5, with the volume increasing gradually from 20 to 60%/day (Hara et al., in press). Water temperatures were 23.8-28.1°C during the rearing period.

In a related experiment on the feeding pattern, 310,000 hatched larvae were stocked in a 5-t concrete tank on 6 November 1984 and reared in the same manner as above. On each of days 9, 15 and 21, 600 larvae were transferred to cylindrical black-painted plastic tanks containing, respectively, 20, 50 and 100 l of water, with mean rotifer density of 10-14/ml, at water temperatures of 26.4-30.3°C. Every hour over 24 hours, illumination in the tank was recorded (Minolta T-1; ± 2% lx), samples of ten larvae were measured and their digestic tracts dissected for rotifer counts. In addition to the hourly samples, ten larvae were sampled every 10 min. between 5 and 6:30 a.m. to monitor feeding during the first break of light.

Results

Siganus guttatus larvae initially fed on rotifers (lorica width about 130μm) at 2.6 mm TL (day 2). The amounts of rotifers/hours increased as a power function of TL in larvae and juveniles (2.6-14.8 mm TL, n = 247). Based on the log-log plots of the maximum amount of rotifers (R) and the TL, a flexion in this relationship occurs at about 7.0-9.5 mm TL, when R suddenly becomes much higher (Fig. 1A).

Siganus guttatus larvae initially fed on artemia nauplii (width 236 μm) at 4.4 mm TL. The maximum number of artemia nauplii in 1 hour increased as a power function of TL in larvae and juveniles (4.4-15.9 mm TL, n = 146). Based on the log-log plot of the maximum amount of artemia nauplii (A) against TL, a change in the relationship occurred at about 7.2 mm, when the rate of increase of A decreased (Fig. 1B).

The percentage of artemia nauplii over rotifers taken by *Siganus guttatus* larvae (3.1-21.1 mm TL, n = 200) is shown in Fig. 2. The larvae showed preference for artemia nauplii over rotifers starting 4.4 mm TL. The nauplii made up an average 19% of prey taken in by larvae up to 8 mm TL. This preference became stronger in larvae 8-9 mm TL and larger, when nauplii constituted an average 73% of prey taken. The preference for artemia is remarkable since the density of artemia was only 1/ml, against that of rotifers at 10/ml.

The feeding incidence (% larvae with rotifers in the gut) and the average number of rotifers in the digestive tract of *Siganus guttatus* during the period 5-6:30 a.m. showed that feeding started (10% incidence and about 1 rotifer/gut) at 4 a.m. in day 9 larvae (3.7 ± 0.3 mm TL, n = 156) and day 15 larvae (5.8 ± 0.9 mm TL, n = 228) and at 3 a.m. in day 21 larvae (7.9 ± 1.2 mm TL, n = 242), when illumination was about 0 lx. Active feeding, when 50% or more larvae had fed, occurred at 6:20 a.m. (878 lx) in day 9 larvae, at 6 a.m. (69 lx) in day 15 larvae, and at 5 a.m. (about 0 lx) in day 21 larvae, with average numbers of rotifers in the digestive tract, respectively, 1.8 (range 0-6), 2.9 (0-15), and 1.5 (0-4).

The per cent feeding incidence and the average number of rotifers every hour during the 24-hour period are shown in Fig. 3 with the measured illumination levels for *Siganus guttatus* larvae of different ages. Satiation, when feeding first reached a plateau in the morning, occurred at 10 a.m. for day 9 larvae, and at 8 a.m. for day 15 and day 21 larvae. Larvae of the same age consumed about equal numbers of rotifers throughout the daylight hours (6 a.m.-6 p.m.) averaging 12 rotifers/larva at day 9, 68 at day 15, and 205 at day 21. Feeding incidence decreased during the evening and became zero at 10 p.m. for larvae of all ages. No feeding occurred until 3-4 p.m. the next day in day 9 and day 15 larvae, but day 21 larvae had a few rotifers in the gut at 11 p.m. Thus there is a clear diurnal pattern in the feeding habits of *S. guttatus* larvae and juveniles in the laboratory. Moreover, the daily feeding period becomes wider with growth.

Discussion

The maximum amount of prey taken increases as a power function of the larval size in *Siganus guttatus* and in several other marine fishes as well (Kitajima et al. 1976; Fukusho 1979). The flexion in the TL-R relationship in *S. guttatus* signifies an increase in the requirement for rotifers by 7.0-9.5 mm TL larvae. Under hatchery conditions, such increase in rotifer intake necessitated doubling the amount of rotifers given to larvae at this stage (Hara et al., in press).

The flexion in the TL-A relationship, i.e., the decrease in slope, is not so easy to explain. It does not seem due to limitation in gut capacity, as the intake of rotifers continues to increase at this time. It may be due to prey size selection by the larvae which seek out and capture the larger (though fewer) older artemia left over from previous days' feeding while ignoring the smaller (more abundant) fresh nauplii. Prey size selection is seen in the preference of larvae for low-density artemia over high-density rotifers. Various studies have shown that fish larvae select larger, though rarer, prey as they grow, presumably because larger prey have much higher energy contents (Hunter 1981).

The observed changes in feeding habits of *Siganus guttatus* all occurred at larval sizes 7-9.5 mm, which coincides well with the full osteological development of the feeding and swimming apparatus at 7-8 mm TL (Kohno et al., this vol.). Similar changes in feeding habits, particularly food preference, have also been shown in other species, although these occurred at different sizes, at >10.5 mm TL in *Pagrus major* (Masumura and Fushimi 1972), and at 5.1-5.5 mm TL in *Oplegnathus fasciatus* (Fukusho 1979).

Siganus guttatus larvae exhibit a diurnal feeding pattern similar to larvae of *Clupea pallasi*, *Paralichthys olivaceus* and *Chanos chanos* (Kurata 1959; Yasunaga 1971; Hara et al. 1983). Vision is important to *S. guttatus* larvae, and the development of the visual apparatus along with the other sense organs with growth probably accounts for the ability of older larvae to feed earlier and for longer periods, as has generally been shown for fish larvae (Kawamura and Hara 1980; Hunter 1981).

Acknowledgement

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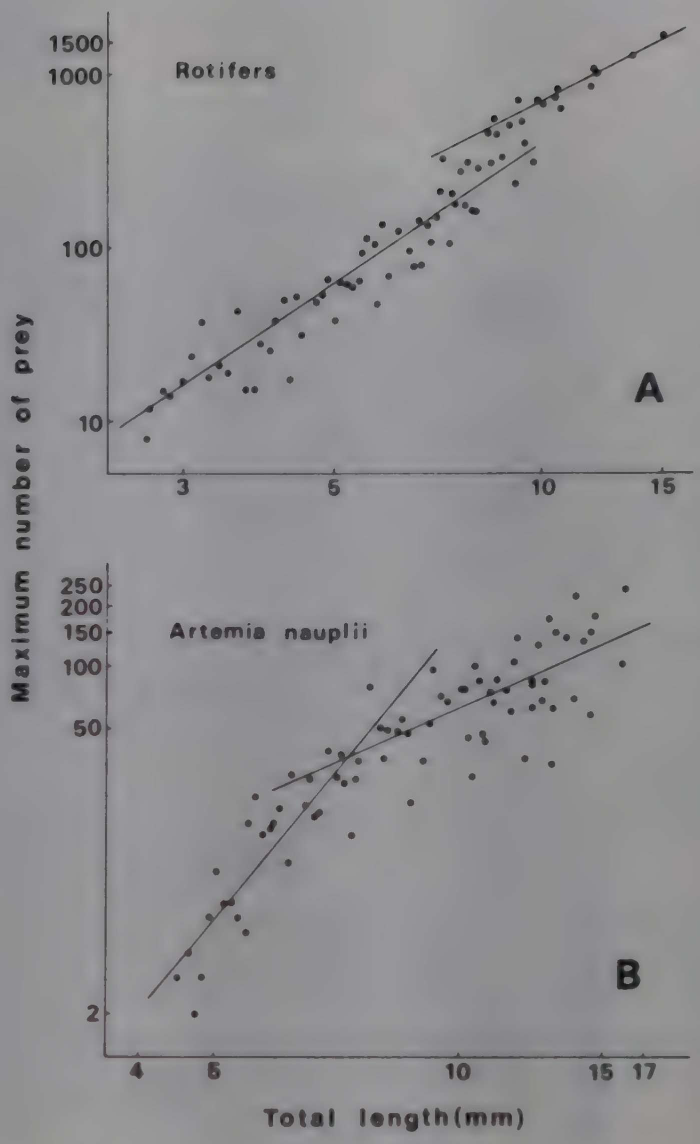

Fig. 1. Maximum number of prey/hr taken by *Siganus guttatus* larvae and juveniles in relation to growth: A, rotifers and B, artemia nauplii.

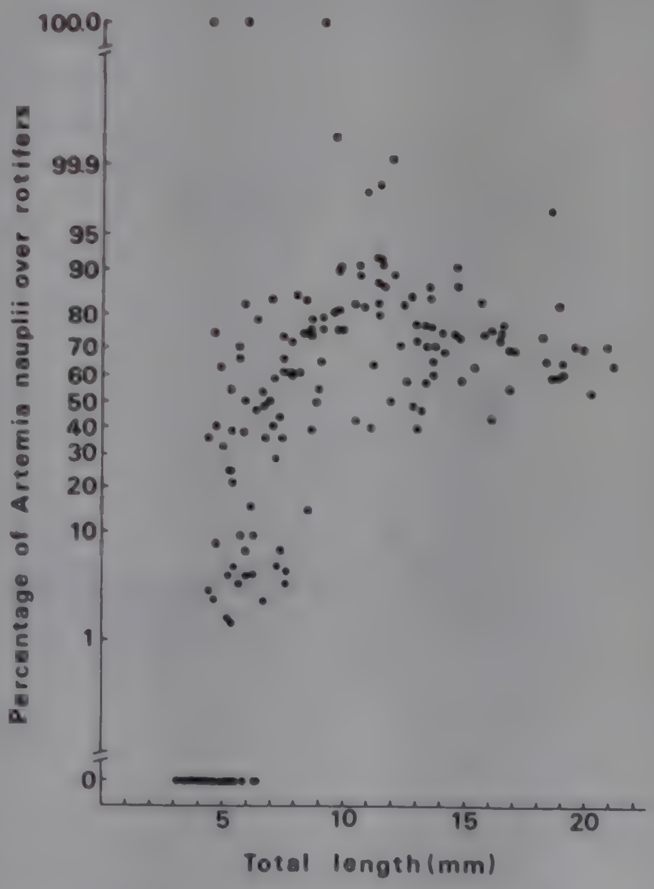

Fig. 2. Preference by *Siganus guttatus* larvae for artemia nauplii over rotifers in relation to growth.

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* SEAFDEC Aquaculture Department Contribution No. 193.

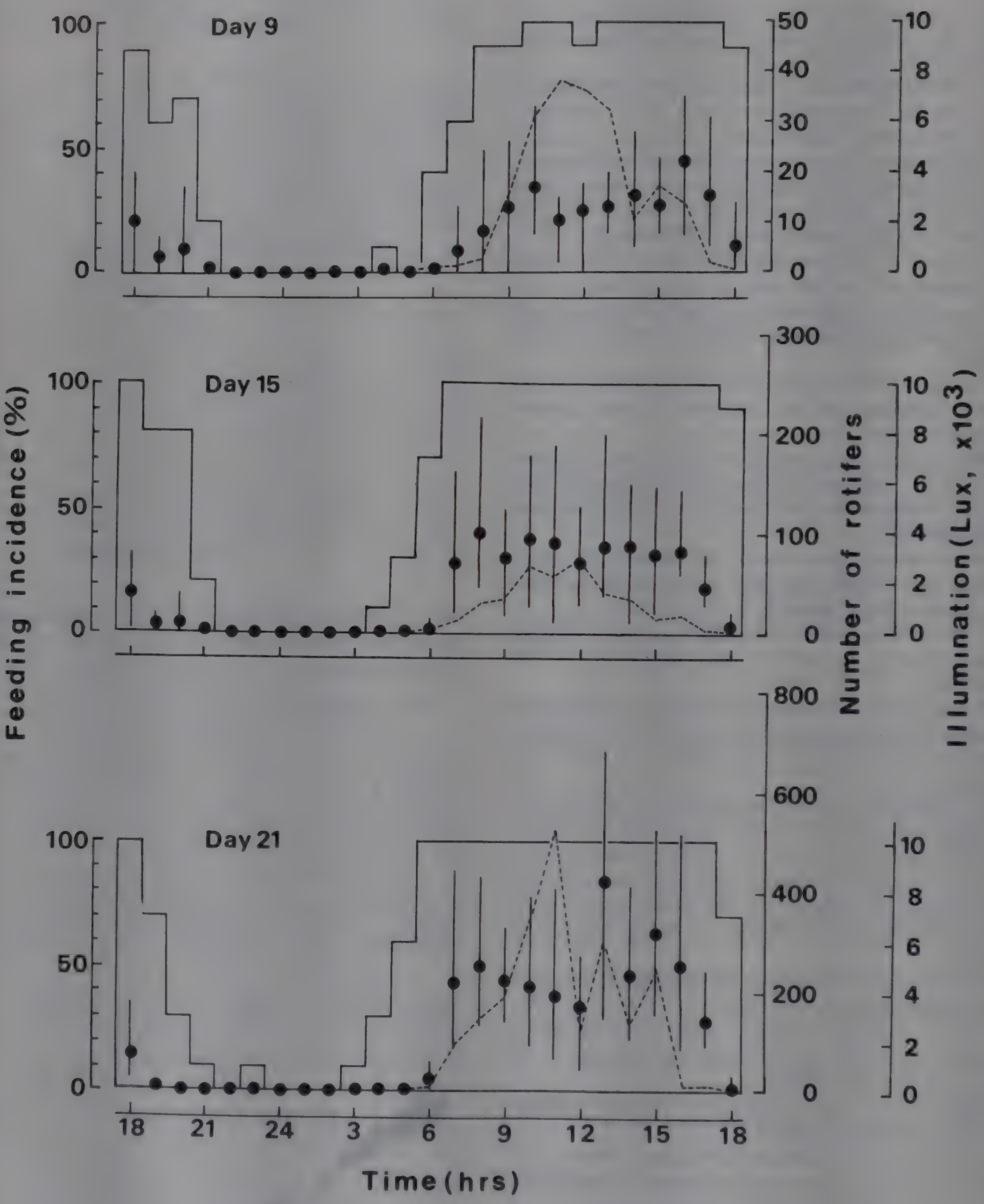

Fig. 3. Hourly change in feeding incidence (% larvae with food in the digestive tract; left ordinate, block space) and in the number of rotifers taken (right ordinate, solid circles with bars representing means and ranges) during a 24-hour period of *Siganus guttatus* of different ages, shown with illumination (broken line).

The Use of Freshwater Hyacinth *Eichhornia crassipes* in Cage Culture in Lake Rawa Pening, Central Java

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Abstract

Rawa Pening is extensively utilized as a hydroelectric, irrigation, fishing and recreational resource. A major constraint to the efficient resource management is the extensive (60%) cover of water hyacinth on this lake. The use of water hyacinth as a feed for cage cultured fish was investigated as a possible solution to this problem. Java carp, *Puntius javanicus;* common carp, *Cyprinus carpio;* tilapia, *Oreochromis mossambicus;* and Sepat Rawa, *Trichogaster* sp., kept in cages suspended in the lake were fed four diets including: 2.5% composted water hyacinth; 10% composted water hyacinth; 2.5% non-composted water hyacinth; and 10% non-composted water hyacinth. All diets were kept at a 25% protein level and contained the same carbohydrate and fat constituents. Feeding frequency was twice a day at 5% body weight per day. Stocking density was $45/m^3$ with an initial stocking weight of 10-20 g. Results showed that in *P. javanicus*, *C. carpio* and *O. mossambicus* substitution of up to 10% by weight of either composted or non-composted water hyacinth in the diets did not significantly decrease the growth rates. *Trichogaster* sp. showed a significantly decreased growth rate at the higher levels of substitution.

Introduction

Rawa Pening, a man-made lake in Central Java, is extensively utilized as a hydroelectric, irrigation, fishing and recreational resource. A major constraint to efficient resource management is the extensive (60%) cover of water hyacinth, *Eichhornia crassipes*, on this lake.

Water hyacinth production came to an estimated 255 t/ha/year in 1976. Water hyacinth in Rawa Pening reduced total fishing production from 398.21 t/year in 1975 to 287 t/year in 1978. Additional water evaporation is estimated to be 2.34 g water per g wet weight of water hyacinth per day (BIOTROP 1978). The indigenous fishes of Rawa Pening are not able to utilize the freshwater hyacinth. Some attempt to control the spread of the weed has been made using chemical and mechanical methods but with limited success.

This study investigates the use of water hyacinth as a possible feed source for cage cultured fish at Rawa Pening. Previous studies had indicated that water hyacinth has between 7 and 13% dry weight protein content; its use as an alternative feed resource would in turn contribute to its environmental control.

Trichogaster sp. (Sepat Rawa) and *Puntius javanicus* (Java carp) were used in this study as representative of indigenous fish and *Oreochromis mossambicus* and *Cyprinus carpio* as introduced species in Rawa Pening (Goltenboth 1978).

Materials and Methods

The cage construction is shown in Fig. 1. The frame was made of bamboo with polystyrene floats and nylon 3-mm mesh netting.

Each species was fed on four diets: Diet I, containing 2.5% dry weight composted water hyacinth; Diet II, containing 10% dry weight composted water hyacinth; Diet III, containing 2.5% dry weight finely cut water hyacinth and Diet IV, containing 10% dry weight finely cut water hyacinth (Table 1). All diets were kept at a 25% protein level and contained the same carbohydrate and fat constituents. Growth measurements for each diet were carried out in triplicate, giving a total of 48 cages. The stocking density in each cage was $45/m^3$. The individual body weights of a ten-fish subsample were measured from each cage once per week. A ration totalling 5% body weight per day was fed twice per day. This ration was calculated on the basis of the previous week's body weight measurements. Specific growth rates of the different species under different diets were calculated from the coefficients of linear regressions of the natural logarithm of the individual fish weights with time (Table 2). Significant difference between coefficients was tested by analysis of variance of the residual variations about the separate and merged regressions (Mead and Curnow 1983).

Results and Discussion

Water temperature remained within the range of 28-30°C and dissolved oxygen of 7.5-9.1 ppm. Increases of mean individual weight with time for the four species are given in Figs. 2-5. Regressions of natural logarithm of weight with time are given in Table 2. *O. mossambicus* exhibited a small but significant decrease in specific growth rate when fed composted and non-composted water hyacinth at the 10% level. *Cyprinus carpio* again showed significant but small decrease in specific growth rate when fed water hyacinth at the 10% level. However, the non-composted form gave significantly better growth rates than the composted water hyacinth. The best growth rate measured in *Puntius javanicus* was in fish fed with the 10% non-composted diet. No significant difference in growth rate was seen in fish fed the 25% and 10% composted water hyacinth diet. *Trichogaster* sp. was the only species to show significant marked decreased growth rate with increase of the proportion of water hyacinth in the diet.

The present results show that both composted and noncomposted processed water hyacinth are suitable ingredients (up to a 10% level) for the carps *Cyprinus carpio* and *Puntius javanicus*. However, it is important to note the severe decrease in specific growth rate in *Trichogaster* sp. when fed at the higher per cent diet. Each species needs to be tested prior to the inclusion of water hyacinth in the diet.

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Table 1. Composition and proximate analysis of experimental diets.

Ingredients (%)	Composted by urea 2% Diet I	 Diet II	Non-composted Diet III	 Diet IV
Composition:				
Water hyacinth	2.5	10.0	2.5	10.0
Fish meal	35.0	35.0	35.0	35.0
Corn meal	10.0	10.0	10.0	10.0
Rice bran	52.5	45.0	52.5	45.0

Proximate analysis (dry weight) (in %)

Diets	Protein	Fat	COH	Ash	Crude fiber
I	28.05	9.01	45.38	11.02	6.54
II	27.86	9.05	45.06	11.09	6.94
III	25.57	9.49	45.51	12.06	7.37
IV	25.51	9.24	45.24	12.24	7.77

Table 2. Regressions of natural log of weight increment with time in *Oreochromis mossambicus*, *Cyprinus carpio*, *Puntius javanicus* and *Trichogaster* sp.

		r^2	SGR[a] % d-1	p[b]	p
Oreochromis mossambicus					
Diet I	lnwt = 0.097t + 3.07	0.76	1.38	< .001	
Diet II	lnwt = 0.084t + 2.98	0.87	1.20		
Diet III	lnwt = 0.094t + 3.16	0.91	1.34	< .001	
Diet IV	lnwt = 0.091t + 3.04	0.97	1.30		
Cyprinus carpio					
Diet I	lnwt = 0.097t + 2.94	0.84	1.38		
Diet II	lnwt = 0.094t + 2.96	0.82	1.34		< .001
Diet III	lnwt = 0.113t + 2.95	0.89	1.61		< .001
Diet IV	lnwt = 0.108t + 2.95	0.84	1.61		
Puntius javanicus					
Diet I	lnwt = 0.089t + 3.07	0.91	1.27		
Diet II	lnwt = 0.088t + 3.04	0.74	1.26		< .001
Diet III	lnwt = 0.067t + 3.03	0.71	0.96	< .001	< .001
Diet IV	lnwt = 0.101t + 2.99	0.69	1.44		
Trichogaster sp.					
Diet I	lnwt = 0.199t + 2.30	0.66	2.84	< .001	
Diet II	lnwt = 0.105t + 2.45	0.29	1.50		
Diet III	lnwt = 0.095t + 2.46	0.32	1.36	< .001	
Diet IV	lnwt = 0.066t + 2.38	0.47	0.93		

[a] SGR = specific growth rate.
[b] p = significant difference between regression coefficients.

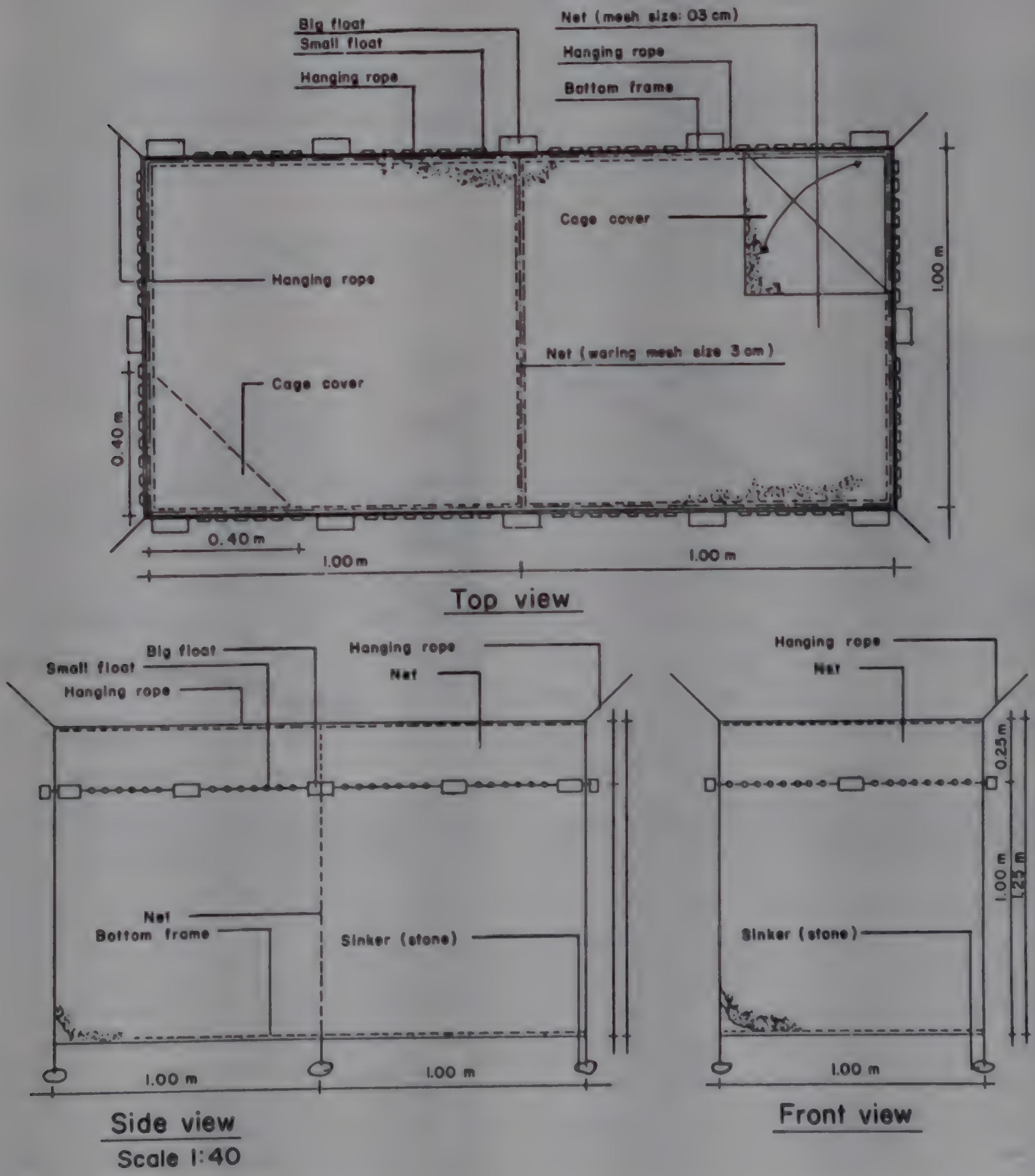
Big float
Small float
Hanging rope
Net (mesh size: 03 cm)
Hanging rope
Bottom frame
Cage cover
Hanging rope
1.00 m
Net (waring mesh size 3 cm)
Cage cover
0.40 m
0.40 m
1.00 m
1.00 m
Top view
Big float
Small float
Hanging rope
Hanging rope
Net
Net
Bottom frame
Sinker (stone)
1.00 m
1.00 m
Side view
Scale 1:40
Hanging rope
Net
0.25 m
1.00 m
1.25 m
Sinker (stone)
1.00 m
Front view

Fig. 1. Cage construction.

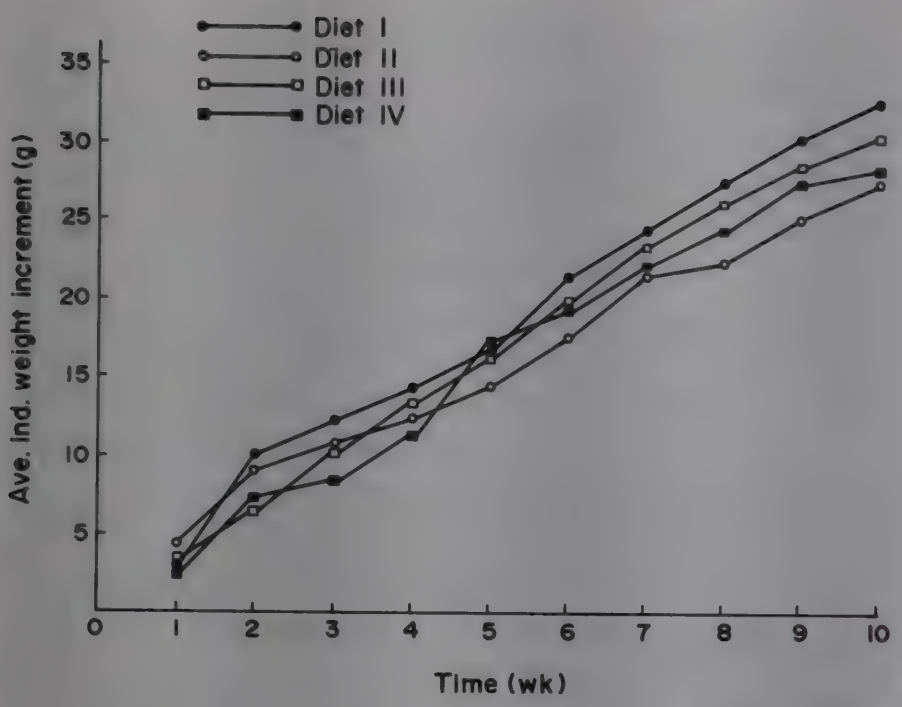

Fig. 2. The relationship of weight increment of *Sarotherodon mossambicus* (g) with time (weeks) in the different diets.

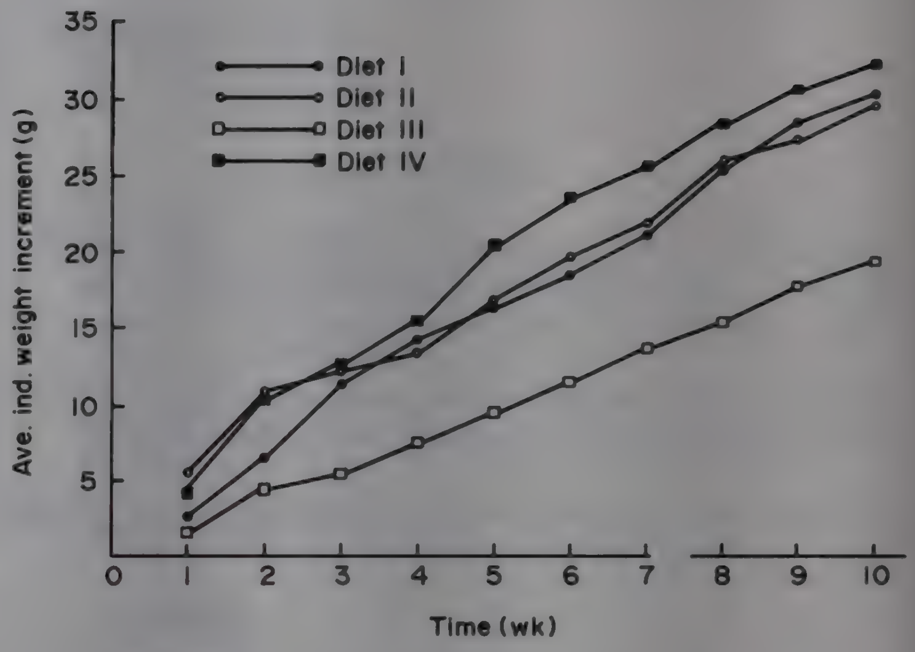

Fig. 4. The relationship of weight increment of *Puntius javanicus* (g) with time (weeks) in the different diets.

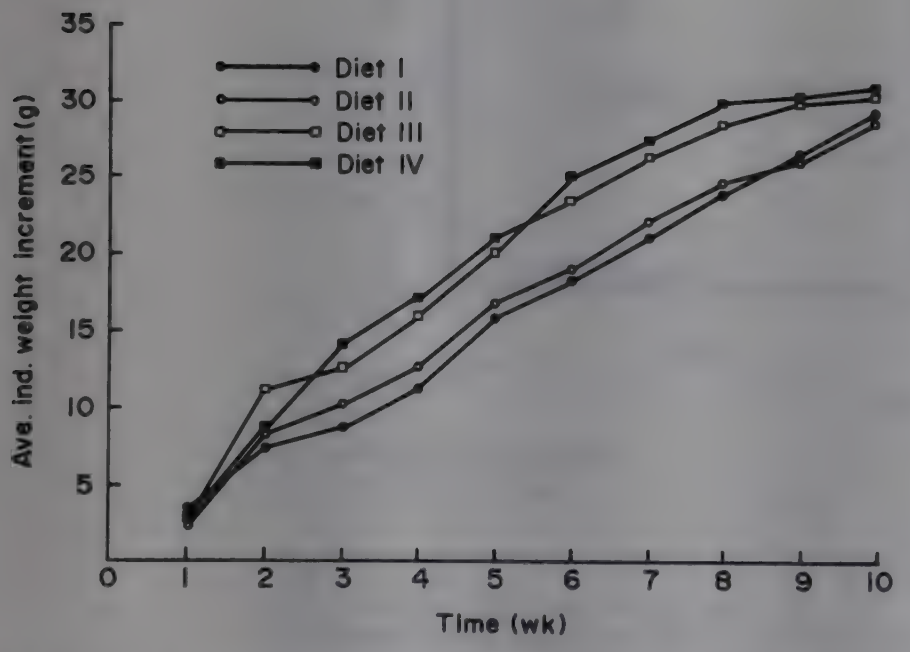

Fig. 3. The relationship of weight increment of *Cyprinus carpio* (g) with time (weeks) in the different diets.

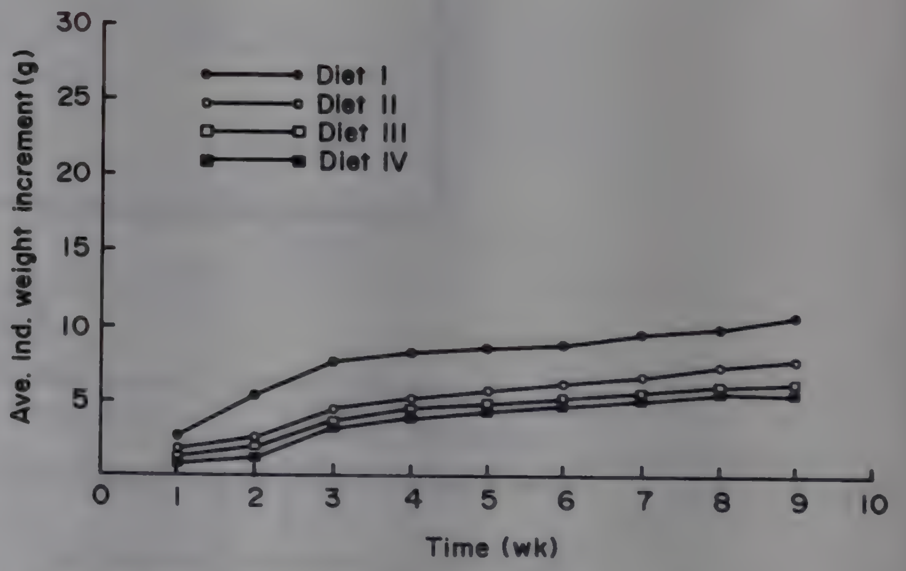

Fig. 5. The relationship of weight increment of *Trichogaster* (g) with time (weeks) in the different diets.

Acetes as Prime Food for *Penaeus monodon* Larvae

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Abstract

Although various artificial shrimp diets have been formulated, they are either too expensive or limited in commercial availability. On the other hand, natural food organisms are difficult to maintain and are often inconsistent in supply. This paper presents research attempts to develop a suitable artificial diet for shrimp larvae with locally-available materials. Fresh and dry *Acetes* are relatively cheap and available in large quantities in tropical waters. Larval rearing experiments using finely ground *Acetes* tissues conducted under various climatic conditions and hatchery systems were completed. In the dry season, larvae in outdoor tanks fed dry *Acetes* had the highest survival rate (68%) compared to larvae fed *Chaetoceros* (48%) or fresh *Acetes* (39%). The larvae in outdoor tanks molted to postlarval stages within eight to nine days. In contrast, larvae from an indoor hatchery reared with *Chaetoceros* had higher survival rate (52%) than those fed with *Acetes* (35%) and fresh *Acetes* (24%); however, the molting period from eggs to postlarvae took 11-12 days.

During rainy months, the survival of larvae reared with *Skeletonema*, dry and fresh *Acetes* in outdoor tanks was 72%, 52% and 38% and in indoor tanks 62%, 40% and 23%, respectively. However, the molting period from eggs to postlarvae was 9-10 days and 12-13 days in outdoor and indoor tanks, respectively.

Introduction

At present, natural food organisms such as *Chaetoceros* sp., *Skeletonema* sp., *Chlorella* sp., *Brachionus* sp. and brine shrimp *Artemia salina* are still the major feed in marine shrimp larval rearing. However, the culture of live food organisms for mass production of shrimp larvae is costly in terms of manpower, infrastructure requirements, equipment and fluctuations in the nutritional value of rotifers and brine shrimp, aside from the fact that their dietary value for shrimp larvae may not be optimal (Simpson et al. 1982). Furthermore, diatoms and other algae can bioconcentrate potentially hazardous chemicals like pesticides from the seawater and sediments.

While it has been possible for many years to feed *Penaeid* postlarvae successfully on artificial diets (Shigueno 1975), problems of nutrient leaching, particle breakdown and water fouling have prevented the use of such diets for early planktonic larval stages. Only recently, it has been demonstrated that the larvae of *Penaeid* shrimps from the first protozoa substage to postlarvae can be reared on a microencapsulated diet (Jones 1984). However, the price of this microencapsulated diet may be high and the supply limited. On the other hand, it has been reported that the exclusive use of crustacean wet tissue suspension as feed for all larval stages is possible (Hameed 1982).

Crustaceans which have been successfully processed into a wet tissue suspension for larval feeding include paste shrimps *Acetes* sp. and *Metapenaeus* sp., stomatopod *Oratosquilla* sp. and mantis shrimp *(Squilla* sp.*)*. The advantages of using crustacean tissue as a larval feed are that the raw materials are locally available and relatively cheap in sufficient quantities. In addition, it is a simple feeding system, which can be easily adopted by small-scale or backyard hatcheries so that the use of live food organisms can be dispensed with. However, there are no reports comparing the effects on survival and growth of postlarvae in relation to seasonal variations and hatchery systems when the larvae are fed on crustacean tissue compared to live food organisms. Thus, this study aimed to determine the possibility of using wet and dry crustacean tissue to replace the live food organisms in different seasons and different hatchery systems.

Acetes is well known as raw material for shrimp paste throughout Asia. It is widely used as animal food for marine finfish larvae because of its high nutritional value which is also required by shrimp larvae. Although there is some seasonal fluctuation in the availability of *Acetes*, dried processed *Acetes* is available cheaply all year round in large quantities.

Materials and Methods

The experiments were conducted in different climatic conditions and hatchery systems. The postlarvae were raised in an outdoor hatchery in a 1-t fiberglass larval rearing tanks exposed to direct sunlight and in an indoor hatchery with 2-t ferrocement larval rearing tanks located in a building under transparent roofing. April to June and August to October represent the dry and rainy seasons, respectively. Three types of feeds were tested; *Chaetoceros* sp. and dried and fresh *Acetes* were used during the dry season, while *Skeletonema* sp. replaced *Chaetoceros* sp. during the rainy season because of unavailability of the latter during that period. Each feed was tested in three replicates in a completely randomized system.

Penaeus monodon nauplii were stocked in larval rearing tanks at 50 and 40/l in outdoor and indoor tanks, respectively, during the dry season while 60/l were used in both types during the rainy season. Salinity, temperature and pH were maintained at 34 ± 2 ppt, 30 ± 2°C and 8.0 ± 0.3 during the dry season and 30 ± 2 ppt, 28 ± 3°C and 8.0 ± 0.3 in the rainy season, respectively. Algal densities were kept at 40,000 live *Chaetoceros* and *Skeletonema* cells/ml. Both algal species were mass produced in TMRL media (Liao and Huang 1973) in 1-t outdoor culture tanks. Prepared dried and fresh *Acetes* in particular sizes were fed four times each day at 0830, 1200, 1700 and 2400 hr based on 10 mgm/larva/day for dry *Acetes* and 50 mgm/larva/day for fresh *Acetes* with 20% increase daily. Table 1 shows the details of the feeding scheme throughout the experiment.

Only 20-30% of the rearing water was changed daily during the protozoea stage and 40-50% during the mysis and postlarval stages.

The condition of the larvae in each tank was observed daily. Larval and algal density, water temperature, pH and salinity were measured. Algal density was adjusted according to survival rate. Outdoor tanks were covered with dark plastic sheets from 1300-0800 hr to maintain the water temperature during the evening and early morning.

One day after metamorphosis to the postlarval stage, the surviving shrimps from each larval rearing tank were counted to determine the overall survival rate and molting.

Fresh *Acetes* were thoroughly washed with water and drip-dried and blended with water (1:1 by volume) for about three min. Blending time depended on the desired particle size of the tissue to be used as food for a particular larval stage.

The blended tissue was passed through a series of nylon sieves of mesh 500μm, 350μm, 250μm, 100μm and 50 μm. Tissue particles that pass through 50μm sieves were used to feed protozoea and bigger sized tissues for larger larvae. Particles retained in the 500μm sieve are discarded as these usually consist of the shell or hard tissues which cannot be blended. The processed tissues were then drip-dried and stored in the freezer until ready for use.

For dry feed, freshly-caught *Acetes* were sundried to remove about 80% of their moisture. Prior to grinding the dry *Acetes* were further dried in an oven for two hours at 60°C. The oven-dried *Acetes* were then ground and sifted through sieves with meshes of 50, 100, 250, 350 and 500 μm. The different particle sizes of ground dried *Acetes* were collected and used as feed according to the larval stage of the shrimp (Table 2).

Results

The proximal analysis values of the *Acetes* used in these experiments are given in Table 3. The results of feeding experiments on feeding in various hatchery systems and climatic conditions (Table 4) indicate that the survival rate of postlarvae fed with dried *Acetes* in outdoor tanks was high (68%) in the dry season. Those fed with *Chaetoceros* sp. in indoor tanks had the highest survival (52%). The survival rates of larvae were significantly different ($p < 0.05$) among the treatments in the same hatchery system. They were also significantly different ($p < 0.05$) in survival rate of the larvae fed with the same food organisms in indoor and outdoor tanks. The larvae fed with fresh *Acetes* had the lowest survival rate - 38.1% and 24% in outdoor and indoor tanks, respectively.

There was a big difference in the length of the molting period from nauplii to postlarval stages between the outdoor and indoor tanks. Only nine days from stocking, the larvae in the outdoor tanks metamorphosed to postlarval stage whereas it took 11-12 days in the indoor tanks.

During the rainy season, survival of larvae fed with *Skeletonema* to postlarval stage was significantly ($p < 0.05$) higher, 72% and 62% in outdoor and indoor tanks, respectively. However, the survival rate of the larvae in outdoor tanks was significantly different ($p < 0.05$) from those in indoor tanks fed with the same food organism.

Survival rates of the larvae in all treatments in the dry season was not statistically higher than those in the rainy season. Survival rate in outdoor tanks was significantly higher than in indoor tanks in the same climatic condition.

The rate of metamorphosis varied in the outdoor and indoor tanks (Table 5). The outdoor tanks benefited from controlled temperatures as the tanks were covered after 1300 hr while the indoor tanks were not covered and were affected by strong winds, especially during the rainy season. *P. monodon* larvae fed with the same type of feed in outdoor tanks had better survival rate and shorter culture period during the dry season.

Discussion

This study provides direct evidence that *P. monodon* larvae can be fed *Acetes* tissue, both in dry and fresh form without any supplementary feeding and are comparable to larvae fed microscopic algae. It was observed that in both cases where fresh and dry *Acetes* were used the tanks developed algal blooms.

The higher rate of metamorphosis in the outdoor tanks is attributed to exposure to direct sunlight, thus to higher water temperature. The tanks were covered when the water temperature was at its highest (31-32°C) at around 1300 hr or 1400 hr with a dark plastic sheet to control the temperature throughout the night. The early morning temperature was still around 29-30°C. In contrast, the temperature in the indoor tanks decreased to 26-27°C in the early morning. This may have affected the metamorphic rate of the larvae. During the dry season, the temperatures were suitable for larval raising without any temperature controls, whereas in the rainy season the temperatures were lower, resulting in slower larval growth and longer molting period.

These experiments also indicate that larvae fed fresh *Acetes* tissue have lower survival rates. It was noted that the larvae fed fresh *Acetes* had red spots on the body when they reached mysis 3, were weak and suffered a high mortality rate. The highest bacteria count was found in the tank fed fresh *Acetes*. The cause is not known.

In terms of survival and growth, feeding *P. monodon* larvae dry *Acetes* can greatly benefit small-scale hatchery operations as the method uses a single food item which is cheap and locally-available in large quantities.

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Table 1 Feeding regimes of *Penaeus* larvae.

	Dry *Acetes*		Fresh *Acetes*		*Chaetoceros* sp. or *Skeletonema* sp. + *Brachionus* sp.
Stage	Size (µm)	Amount (mg/larva/day)	Size (µm)	Amount (mg/larva/day)	(cells/ml)
Nauplii	less than 50	10.0	less than 50	50.0	40,000
Protozoea I	less than 50	12.0	less than 50	60.0	40,000
Protozoea II	less than 50	14.2	less than 50	72.0	40,000
Protozoea III	50-100	17.0	50-100	86.4	40,000
Mysis I	50-100	20.4	50-100	103.7	40,000 + 3 – 5 *Brachionus*
Mysis II	100-250	24.5	100-250	124.4	"
Mysis III	100-250	29.4	100-250	149.2	"
Postlarva I	250-350	35.2	250-350	179.0	"

Table 2. Sizes of feed particles used for various larval stages of *P. monodon*.

Particle size (µm)	Larval stage
Less than 50	Protozoea I, II
50- > 100	Protozoea III, Mysis I
100- > 250	Mysis II, III
250- > 350	Early postlarvae

Table 3. Proximal composition of *Acetes*.

Composition	%
Crude protein	54.46
Crude fat	3.74
Crude fiber	4.88
Nitrogen free extract	21.72
Ash content	15.20
Calcium	3.44
Phosphorus	1.25

Table 4. Feeding schemes in different hatchery systems and seasons.

Season	Hatchery	Treatment	No. of run	Average amount of animal/run Nauplii	Protozoea	Mysis	PL_1	Molting period	Survival rate $N-PL_1$
Dry[a]									
	Outdoor[a]	A	4	52,000	46,000	38,000	35,000	9	68%±14.8[a]
		B	4	52,000	41,000	30,000	25,000	9	48%±15.1[b]
		C	4	52,000	41,000	29,700	19,500	9	38%±18.7[c]
	Indoor[b]	A	4	82,000	62,000	35,000	28,700	11-12	35%±10.1[y]
		B	4	82,000	64,000	48,000	42,700	11-12	52%±15.0[x]
		C	4	82,000	62,000	32,000	20,000	11-12	24%±15.3[z]
Rainy[b]									
	Outdoor[a]	A	3	62,000	53,000	42,000	32,500	9-10	52%±13.6[a]
			3	62,000	54,000	48,000	45,000	9-10	72%±10.3[b]
			3	62,000	52,000	38,000	23,500	9-10	38%±14.3[c]
	Indoor[b]	A	3	120,000	92,000	68,000	48,000	12-13	40%±9.0[x]
			3	120,000	92,000	82,000	74,500	12-13	62%±9.9[y]
			3	120,000	86,000	32,000	27,600	12-13	23%±10.3[z]

Treatment A, Dry *Acetes;* B, *Chaetoceros* sp.; C, Fresh *Acetes;* and D, *Skeletonema* sp.

Note: Items followed by the same superscript are not significantly different at $p < 0.05$.

Table 5. Metamorphosis of *P. monodon* larvae to one-day old postlarvae.

	Days after stocking 1	2	3	4	5	6	7	8	9	10	11	12	13
Dry													
Outdoor	N	$N-Z_1$	Z_1	Z_2	Z_3	M_1	M_2	M_3	P_1				
Indoor	N	$N-Z_1$	Z_1	Z_1-Z_2	Z_2	Z_2-	Z_3	M_1	M_2	M_3	M_3-P_1	P_1	
Rainy													
Outdoor	N	$N-Z_1$	Z_1	Z_1-Z_2	Z_2-Z_3	Z_2-M_1	M_1-M_2	M_2-M_3	M_3-P	P			
Indoor	N	$N-Z_1$	Z_1	Z_1-Z_2	Z_2	Z_2-Z_3	Z_3	Z_3-M_1	M_1	M_1-M_2	M_2-M_3	M_3-P_1	P_1

N = nauplii; Z = zoea; M = mysis; P = postlarva.

Effect of Dietary Protein Levels on Ovarian Maintenance and Reproductive Performance of the Adult Dwarf Goramy, *Colisa lalia* (Hamilton)

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Abstract

Five experimental diets formulated to contain 5, 15, 25, 35 and 45% protein were prepared using casein and fishmeal as protein sources. These diets were fed to adult female dwarf goramys stocked in glass tanks. After 20 weeks of feeding, fish from each dietary treatment were paired with males. The number of fish spawned, eggs spawned and fry hatched (reproductive performance) were recorded. Ovary samples were also taken from females from each of the five treatments.

Results indicated that the fish fed high protein diets (25, 35 and 45%) had higher mean body weight, ovary weight and reproductive performance than those fed the low protein diets (5 and 15%). There was no significant difference among the high protein diets nor between the two low protein diets. The low protein diets also showed a greater decline in the percentage of yolky oocytes than the high protein diets.

Introduction

The quantity and nature of dietary proteins are known to affect fish reproduction. For example, in the guppy, decreased dietary protein levels resulted in decreased ovary weight and volume although fecundity was not significantly affected (Dahlgren 1980). However, in the red sea bream, *Chrysophrys major*, fecundity and egg hatchability were affected by the nature of the protein component in the diet of the female broodstock (Watanabe et al. 1984). Little work has been published on the relationship of diet to ovarian maintenance and reproductive performance in the adult female fish.

Materials and Methods

Adult female goramys approximately 4 months old were purchased from a local fish farm. The fish were maintained in five identical glass aquaria of 60 l each. At the beginning of the experiment 60 fish were stocked in each tank. Fish were sampled at regular intervals and their weights were recorded. The temperature of the tank water remained at 27 ± 2°C and the pH remained near 7.

Fishmeal and casein were the dietary protein sources. They were blended with dextrin, corn oil, tapioca flour, cod liver oil, and a vitamin and mineral premix to prepare diets with 5, 15, 25, 35 or 45% protein (Shim and Chua 1983). The composition of these diets is given in Table 1. All diets were formulated to be isocalorific. The diets were kept refrigerated until needed. The fish were fed 3% of their body weight twice daily six days/week. A natural 12-hour day and night photoperiod was maintained.

After 20 weeks, five female fish were taken from each tank and paired with males from the farm. In addition, four females were taken from each tank and dissected to study their ovarian condition. The number of oocytes at each stage of development was recorded at the beginning and end of the experiment. The criteria used by Teo (1984) were used to separate the oocytes into different stages.

Results

Changes in body weight in the fish fed the five experimental diets are shown in Table 2. Among the different dietary treatments the low protein (5 and 15%) treatments showed a significant loss of weight at the end of the experiment while the high protein (25 to 45%) diet fish showed a significant weight gain after 20 weeks. The 35% protein diet produced fish with the greatest mean weight at the end of the 20-week experimental period. There was no significant difference in the mean weights of fish from the 25 to 45% protein diets. These results indicate that raising the level of dietary protein up to 25% increased the weight gain in female goramys. However, increases in the dietary protein level beyond 25% did not give significantly better weight gains in these fish.

The effect of a low protein diet on the distribution of oocytes in the different developmental stages is shown in Table 3. The highly significant values of X^2 demonstrate that there is an effect of dietary protein level on oocyte distribution. There was a decrease in the percentage of yolky oocytes at the end of 20 weeks in all of the five dietary treatments, but the low protein treatments (5 and 15%) showed a greater decline.

Further evidence to support the adverse effect of low protein diets on ovarian maintenance in females is given in Table 4. The low protein fish had significantly lower mean ovary weights than those fed on high protein diets. The same relationship holds true for mean gonadosomatic index (GSI).

The results of spawning trials are summarized in Table 5. Although the dietary protein level had no statistically significant effect on the percentage of fish spawned, the number of eggs hatched, or the number of larvae produced, the respective values appeared to be higher for the high protein diets. Although the males were obtained from the farm, their effectiveness in fertilizing the eggs may have differed. This male variability may have obscured the effect diet had on female reproductive performance.

Discussion

Of all the major ingredients used to formulate fish feed, protein is the most expensive. Therefore when practical diets are formulated it is desirable to provide the minimum amount of protein necessary for good growth and feed conversion (Nose and Arai 1972). When broodstock is cultured, however, optimum growth is not enough. Attention must be paid to the effect of protein level on ovarian maintenance and reproductive performance. This experiment has shown that in the dwarf goramy high protein diets are necessary to maintain reproductive performance and preserve ovarian condition in female goramy broodstock.

Although low protein diets did not have a statistically significant effect on percentage females spawning, number of eggs spawned or number of larvae hatched, the data suggest that low protein females do tend to have a smaller production of eggs and larvae (Table 5).

Examination of oocyte distribution, ovary weight and GSI also confirmed the harmful effect low protein diets had on ovarian maintenance in female broodstock. Low protein (5 and 15%) diets produced females with significantly lower ovary weights and GSI than high protein females (25, 35 and 45%). In addition, these low protein females had lower percentages of oocytes in the yolk granule stages (Table 3). Since all fish initially showed predominantly yolk-granule oocytes in their ovaries, these findings suggest that the low protein diets caused a greater degree of ovarian atresia and/or a slower development of the next batch of oocytes compared to the high protein diets. Finally, females fed low protein diets suffered a decrease in their mean body weights after 20 weeks of feeding. These results show that female broodstock require a high protein diet (25% or above) to maintain good reproductive performance.

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Table 1. Composition of experimental diets (g/100 g feed).

Ingredients	% protein in diet 5	15	25	35	45
Casein	1.42	8.58	20.07	31.57	43.07
Fishmeal	7.25	15.25	16.75	18.25	19.75
Dextrin	56.83	44.67	34.68	24.68	14.68
Corn oil	7.50	6.50	5.50	4.50	3.50
Cod liver oil	2.00	2.00	2.00	2.00	2.00
Tapioca	5.00	5.00	5.00	5.00	5.00
Vitamin* premix	1.00	1.00	1.00	1.00	1.00
Mineral* premix	17.00	15.00	13.00	11.00	9.00

*From Shim and Chua (1983).

Table 2. Effect of dietary protein level on goramy weights in grams.

Time (weeks)	Dietary protein level 5%	15%	25%	35%	45%
2	2.79 ± 0.25^{*}	3.04 ± 0.02^{a}	2.76 ± 0.01^{a}	2.88 ± 0.09^{*}	2.92 ± 0.92^{a}
6	2.50 ± 0.14^{a}	2.67 ± 0.10^{a}	2.61 ± 0.05^{a}	3.18 ± 0.22^{b}	2.68 ± 0.06^{a}
9	2.45 ± 0.14^{c}	2.57 ± 0.06^{bc}	2.68 ± 0.07^{bc}	3.02 ± 0.17^{a}	2.88 ± 0.10^{ab}
13	2.34 ± 0.07^{d}	2.54 ± 0.10^{cd}	2.78 ± 0.11^{bc}	3.25 ± 0.12^{a}	3.01 ± 0.12^{ab}
17	2.13 ± 0.09^{c}	2.30 ± 0.07^{c}	2.84 ± 0.12^{b}	3.60 ± 0.14^{a}	2.78 ± 0.15^{b}
19	2.24 ± 0.09^{c}	2.10 ± 0.06^{c}	2.84 ± 0.11^{b}	3.18 ± 0.13^{a}	2.67 ± 0.09^{b}
20	2.31 ± 0.13^{c}	2.29 ± 0.11^{c}	2.78 ± 0.18^{b}	3.22 ± 0.15^{ab}	2.82 ± 0.15^{ab}
Sample size (n)	31	31	30	35	31

Means with the same superscript are not significantly different.

Values are mean body weights ± standard errors.

Table 3. Effect of dietary protein level on change in oocyte distribution over time in goramy ovaries.

Weeks	Oocyte stage	Protein level 5%		15%		25%		35%		45%		x^2
2	PE	30*	(7.5)	44*	(6.9)	68*	(13.4)	51*	(12.2)	43*	(16.0)	40.35**
	YV	35	(8.7)	25	(3.9)	29	(5.7)	34	(8.1)	17	(6.3)	
	YG	337	(83.8)	556	(89.2)	412	(80.9)	334	(79.7)	209	(77.7)	
	Total	402		635		509		419		269		
20	PE	53	(53.5)	456	(51.6)	111	(19.3)	97	(22.9)	118	(30.8)	
	YV	4	(4.0)	53	(6.0)	148	(25.7)	40	(9.5)	24	(6.3)	328.88**
	YG	42	(42.5)	374	(42.4)	316	(55.0)	286	(67.6)	241	(62.9)	
	Total	99		883		575		423		383		

* Numbers listed are total number of oocytes.

( ) Numbers within parentheses are percentages of total number of oocytes.

** p 0.01.

n = 4 ovaries per protein level.

PE = oocytes in perinculeolar stage.
YV = oocytes in yolk vesicle stage.
YG = oocytes in yolk granule stage.

Table 4. Dietary protein effect on ovary weight and GSI (mean weight at end of experiment).

	Protein level					
	5%	15%	25%	35%	45%	Probability
Mean ovary weight	0.0782^b (0.0252)	0.0710^b (0.0233)	0.2790^a (0.0291)	0.3375^a (0.0259)	0.1475^b (0.0309)	$P < 0.001$
GSI	3.24^b (0.92)	3.32^b (0.79)	9.48^a (0.91)	10.309^a (0.72)	5.26^b (1.02)	$P < 0.001$
n	20	20	20	20	20	

Means with the same superscript are not significantly different.
Numbers in brackets are standard errors.

Table 5. Effect of dietary protein level on reproductive performance in dwarf goramy.

Protein level (%)	No. of fish spawned	% fish spawned	No. of unhatched eggs*	Larvae*	Total reproductive output*	% eggs hatched*
5	2	40	25.8 ± 15.1	67.6 ± 338.6	93.4 ± 53.7	29.1 ± 15.9
15	3	60	84.6 ± 62.9	223.6 ± 84.9	308.2 ± 117.1	46.0 ± 18.1
25	4	80	102.6 ± 338.3	253.2 ± 75.5	355.7 ± 101.9	57.8 ± 13.7
35	5	100	206.4 ± 71.4	319.6 ± 89.4	526.0 ± 85.1	61.3 ± 13.6
45	4	80	111.8 ± 72.0	282.0 ± 101.1	393.8 ± 90.4	55.9 ± 25.0

*Values are means ± standard errors.

Suitable Diet and Optimum Feeding Frequency in the Eyestalk Ablated Prawn, *Macrobrachium lamarrei*

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Abstract

Diets consisting of *Tubifex* worm, goat liver and carrot were given to the destalked and normal prawns, *Macrobrachium lamarrei*. *Tubifex* supported maximum growth, especially in destalked prawns. Ablated prawns required higher dietary protein (40%) than non-ablated prawns (36%). To fix an optimum feeding frequency, the prawns were fed at different feeding regimes: once in three days, once in two days, once a day, three times a day, six times a day and 12 times a day. Proffering six or three meals a day supported maximum growth in both normal and ablated prawns.

Introduction

The technique of eyestalk ablation is one of the means of improving prawn production (Rao et al. 1973; Mauviot and Castell 1976) as it leads to increased molting frequency and growth. Smith (1940) ascribed heavy mortality after destalking to imbalanced diet. Feeding with live food, *Artemia salina*, and other synthetic diets of high protein content could improve the survival and growth of *Homarus americanus* (Mauviot and Castell 1975). Economic production of the prawn depends on feeding the best possible diet at an optimum frequency because among the environmental variables, food is considered the most potent factor affecting growth (Kinne 1960). The daily ration of a prawn is influenced by a number of factors, of which the amount of food consumed, the number of meals per day and the rate of gastric evacuation are important. Increasing feeding frequency (Ff) has been found to enhance growth of fishes (Marian et al. 1981). However, there is an optimum which economizes the feed cost and maximizes growth. In this study, a suitable diet and an optimum feeding frequency were selected for the prawn *Macrobrachium lamarrei*.

Materials and Methods

Individuals of *Macrobrachium lamarrei* (H. Milne Edwards) were collected from the Grant Anicut, Tiruchirapalli, for two series of experiments. The first series was designed to select a suitable diet and the second to know the optimum feeding frequency. The prawns were reared individually in a running water system, with temperature of 28 ± 1.8°C. In *Macrobrachium lamarrei*, the intermolt period lasts for 18 days. Since the nutrient input during the intermolt period along with the hormones related to eyestalk ablation determine the successive growth and molting, these studies are restricted to a single molting period.

Healthy individuals of *M. lamarrei* weighing 0.4 ± 0.05 g were selected. One group served as control and another group was destalked. Destalking was performed by cutting the eyestalk at its base (unilateral ablation) with a fine pair of dissecting scissors and the wound cauterized immediately with a hot blunt needle. Both groups were fed *ad libitum* on one of the diets containing different protein levels: *Tubifex tubifex* (60%), goat liver (41%) and carrot (10%).

Similarly, the test individuals were assigned to one of the six feeding frequencies (Table 1). Prawns were fed *ad libitum* with *Tubifex tubifex* for one hour. Care was taken to collect the remaining unconsumed food with a pipette without disturbing the prawn. The aquaria were kept under illumination from 9 a.m. to 8 p.m. During the hours of darkness feeding and collection of unconsumed food were made with a dimlight (0 watt). The 'sacrifice method' (Maynard and Loosli 1962) was used to determine the growth of prawn. Water content of the prawn was determined by taking wet and dry weights of the test individuals. Caloric value was by a Parr 1412 semi-microbomb calorimeter, following the standard procedure described in the instruction manuals nos. 128 and 130 for Parr bomb calorimetry. The bioenergetic parameters were calculated following IBP formula of Petrusewicz and MacFadyen (1970).

Results

Destalked *M. lamarrei* consumed significantly ($P < 0.05$) more food than the normal one. Among the three diets, liver was consumed almost two times more than carrot or *Tubifex* (Table 2). But the percentage of food energy digested and absorbed was the highest (95%) for *Tubifex*, lowest (61.5%) for carrot and moderate (88.5%) for liver. Absorption efficiency was not influenced by eyestalk ablation but food quality. In all the three feeds, the ablated prawn exhibited significantly ($P < 0.01$) faster specific growth rate (Table 2).

In terms of live weight, all prawns exhibited positive growth. But in terms of energy, those fed on the carrot exhibited negative growth (Table 2). Although the ablated group consumed more food than the non-ablated prawns when fed on a low nutrient diet like carrot, the high negative growth may be due to the expenditure of a greater proportion of energy on metabolism.

The *Tubifex*-fed group not only absorbed more food (95%) but also converted it into energy with higher efficiency (20%). Although more liver was consumed, the prawn could just maintain its body weight and exhibited very low growth efficiency (K_1 = 0.06-1.43%). Conversion efficiency of ablated prawn was significantly ($P < 0.05$) higher than the non-ablated one which clearly shows that feeding *Tubifex* to destalked prawn is advantageous.

Food consumption per meal gradually increased with decreasing Ff up to once in a two-day feeding regime in both the ablated and normal groups. Further decrease in Ff did not increase the meal size (Fig. 1). To relate food consumption to the amount of food remaining in the stomach, experiments on gastric evacuation were undertaken (Fig. 2). Destalking resulted in hyperphases and the prawn consumed the meal to full stomach capacity (12.2% body weight), while normal prawn food consumption was only 7.3% body weight. The rate of gastric evacuation was higher (9.8×10^{-2}) in the destalked prawn than control (6.8×10^{-2}). In both groups, *M. lamarrei* consumed maximum food in a meal when the food deprivation was 48 hours. Eyestalk ablation appeared to regulate the stomach capacity. While Ff or deprivation time controlled the meal consumption, the maximum food intake at the time of stomach evacuation was 90%. With increasing Ff, the food consumption increased from 143 to 2,398 J/g/day in the control group, while in the destalked groups the levels of food consumption were significantly higher ($P < 0.001$) (Table 3). Absorption efficiency of *M. lamarrei* observed in control as well as in destalked groups averaged 94%. The minor variations observed among the groups were not significant ($P > 0.05$) (Table 3). All the groups both in control as well as ablated *M. lamarrei* exhibited positive growth, while the lowest Ff group (Ff-1/3) in either cases exhibited negative growth. The rate of negative growth in the ablated group was half of that exhibited by the controls (Table 3).

Food conversion efficiency was low at the lowest feeding level and gradually increased as the rates of feeding increased and declined at the highest levels in both groups. In the ablated group the maximum conversion efficiency was 22.3% in those receiving food thrice a day, while in the control group it was (20.5%) in that fed once a day. Considering the rates of production in the destalked prawns, the Ff 3/1 was found to be the optimum, as it maximized the growth rate (296 J/g/day). But in the control groups, the highest growth rate was observed in those fed 12 times a day. When both growth rate and feed cost are considered, three times a day is the optimum for the destalked prawn but six times a day for the controls. The techniques of destalking and culturing destalked *M. lamarrei* not only increased production but also minimized the feed cost.

Discussion

Eyestalk ablation in decapods has been shown to result in more frequent molting, accelerated growth and greater egg output, e.g., *Panulirus homarus* (Radhakrishnan and Vijayakumaran 1984a, 1984b). Our observations in *Macrobrachium lamarrei* confirm these reports. Ablated decapods procure additional energy to sustain frequent molt, accelerated growth efficiency (Pandian and Kumari 1985). Ablated *M. lamarrei* has also been observed to increase in feeding rate and enhance growth efficiency. Hence, it is likely that eyestalk ablation may be used as a technique to increase prawn production in aquaculture.

Ablated decapods are known to mobilize a greater amount of protein for energy metabolism. Consequently, the protein requirement in the ablated prawns may be expected to go up. Mauviot and Castell (1976) observed accelerated growth in ablated *Homarus americanus* when fed protein-rich diet (60%). In *M. lamarrei*, too, ablated prawns grew faster when fed *Tubifex* rather than carrot.

As already indicated, food requirements of ablated decapods are higher than those of a control. Accelerated digestion and faster stomach evacuation observed in ablated *M. lamarrei* appear to return the appetite quickly. Hence, ablated animals require more food and grow faster at higher efficiency when fed three times a day, whereas the control animals sustained normal growth receiving food once a day.

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Table 1. Feeding schedule of *Macrobrachium lamarrei.*

Feeding frequency (Ff)	Notation	Time (hr) 1	2	3	4	5	6	7	8	9	10	11	12
12x a day	12/1	06.00	08.00	10.00	12.00	14.00	16.00	18.00	20.00	22.00	24.00	2.00	4.00
6x a day	6/1	06.00	–	10.00	–	14.00	–	18.00	–	22.00	–	2.00	–
3x a day	3/1	06.00	–	–	–	4.00	–	–	–	22.00	–	–	–
1x a day	1/1	06.00	–	–	–	–	–	–	–	–	–	–	–
1x 2 days	1/2	06.00	–	–	–	–	–	–	–	–	–	–	–
1x 3 days	1/3	06.00	–	–	–	–	–	–	–	–	–	–	–

Table 2. Effect of food quality on specific growth rate* and bioenergetic parameters of *Macrobrachium lamarrei.* Values represent the average performance of 6 individuals; rates are expressed in J g^{-1} day^{-1}, efficiencies in %.

Food	Condition	Sp. growth rate	Cr	Pr	Mr	Ae	K_1
Tubifex	Ablated	0.532 ± 0.025	797 ± 45	195 ± 6	570 ± 35	96 ± 2	24.5 ± 0.75
	Non-ablated	0.391 ± 0.027	666 ± 30	132 ± 12	534 ± 24	94 ± 2	19.8 ± 1.8
Liver	Ablated	0.355 ± 0.037	1,358 ± 93	0.8 ± 0.32	1,208 ± 81	89 ± 3	0.06 ± 0.02
	Non-ablated	0.263 ± 0.020	1,167 ± 54	16.7 ± 0.94	1,009.3 ± 46	88 ± 2	1.43 ± 0.080
Carrot	Ablated	0.218 ± 0.015	616 ± 49	−1,130 ± 4.32	489 ± 34.32	61 ± 3	–
	Non-ablated	0.124 ± 0.005	524 ± 58	−91.3 ± 3.68	416.3 ± 39.68	62 ± 1	–

*Calculated using the formula $= \frac{\text{lnWt1} - \text{lnWto}}{\text{t1} - \text{to}}$

Cr – consumption rate; Pr – production rate; Mr – metabolic rate; K_1 – gross conversion efficiency. Values are averages ± s.d.

Table 3. Effect of feeding frequency on the bioenergetic parameters of ablated and non-ablated *Macrobrachium lamarrei*. Each value represents the average ± s.d. of six individuals. Rates are expressed in J g^{-1} day^{-1}; efficiencies in %.

Ff	Condition	Cr	Pr	Mr	Ae	K_1
12/1	Ablated	2,560 ± 105	263 ± 10	2,143 ± 98	94 ± 1.58	10.3 ± 1.51
	Non-ablated	2,398 ± 75	235 ± 12	1,995 ± 70	96.2 ± 1.5	9.8 ± 1.58
6/1	Ablated	2,119 ± 41	265 ± 6	1,706 ± 34	93 ± 2.1	12.5 ± 1.45
	Non-ablated	1,036 ± 68	196 ± 14	779 ± 61	94.8 ± 2.3	18.9 ± 2.41
3/1	Ablated	1,327 ± 56	296 ± 18	965 ± 42	95 ± 1.58	22.3 ± 1.91
	Non-ablated	768 ± 65	141 ± 14	572 ± 38	93.4 ± 1.75	18.3 ± 1.53
1/1	Ablated	495 ± 48	74 ± 6	386 ± 48	93 ± 1.74	15.0 ± 1.05
	Non-ablated	302 ± 41	62 ± 4	223 ± 36	94.3 ± 0.85	20.5 ± 1.81
1/2	Ablated	217 ± 15	5 ± 1.3	203 ± 18	96 ± 0.85	2.3 ± 0.2
	Non-ablated	225 ± 25	27 ± 5	163 ± 20	94.1 ± 1.51	12.0 ± 0.58
1/3	Ablated	177 ± 12	−8.0 ± 1.5	174 ± 10	94 ± 1.53	–
	Non-ablated	143 ± 23	−19 ± 3	153 ± 10	94.5 ± 0.91	–

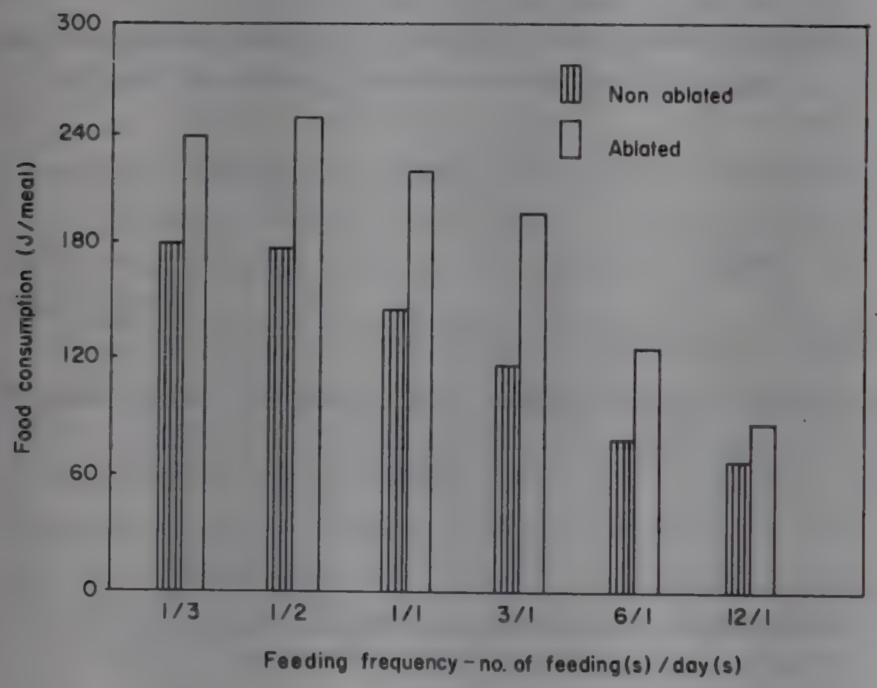

Fig. 1. Relationship between feeding frequency and food consumed by *M. lamarrei*.

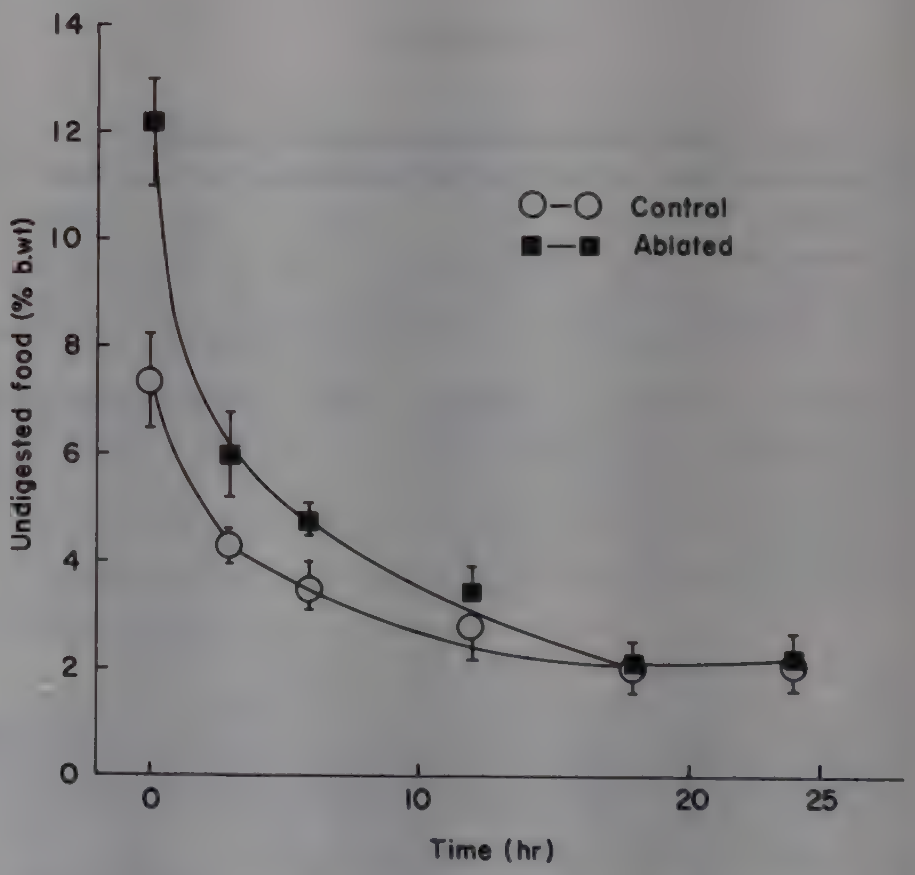

Fig. 2. Relationship between time and stomach content of *M. lamarrei*.

The Effect of Diet on the Reproductive Performance of Pond-Reared *Penaeus monodon* Fabricius Broodstock

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Abstract

Three practical diets were tested for their effects on the reproductive performance, survival and larval quality of pond-reared *Penaeus monodon*. Diets A, B and C were formulated to contain the same basal components but supplemented with different sources of lipids. Lipid sources were cod liver oil (Diet A), soybean lecithin (Diet C) or their 1:1 combination (Diet B). An all-natural diet consisting of squid and marine annelids served as control. Pond-raised *P. monodon* were stocked in four 12-m^3 flow-through maturation tanks with 28 females and 22 males per tank. Broodstock were acclimated to the diets prior to unilateral ablation of females. Reproductive performance in terms of total number of spawnings, eggs and nauplii production, average hatch rate of eggs and larval quality was best for Diet A followed by Diet C. Diet B gave the poorest overall response but was better than the control. In the control most of the mature females resorbed their ovaries and failed to spawn; survival rates of females was also lowest. The results suggest that nutritional quality of broodstock diet affects reproduction and larval survival. Diet A (cod liver oil supplemented) was found to be a suitable diet for successful maturation and spawning of pond-reared *P. monodon*.

Introduction

The giant tiger prawn, *Penaeus monodon* Fabricius, is a commercially important penaeid species in the Philippines and other countries in Asia. Despite the success in its induced maturation and spawning by unilateral eyestalk ablation, controlled reproduction of captive broodstock remains a constraint to commercial production. Current research on penaeids has placed emphasis on proper dietary regimes for optimum reproduction and progeny survival. The nutrition of broodstock is shown to have a considerable effect upon gonadal growth and fecundity, egg and larval quality.

The significance of lipids and polyunsaturated fatty acids (PUFA) in maturation diets for prawn has been reported by several investigators (Lawrence et al. 1979; Middleditch et al. 1979). Studies using a formulated diet that simulate the fatty acid profile of wild *P. monodon*, at the SEAFDEC Aquaculture Department, have shown the importance of PUFA in reproduction and revealed correlations between broodstock reproductive performance and fatty acid patterns of the diet (Millamena et al. 1985a). The use of marine worms, molluscs and crustaceans in prawn maturation diets is based on the high levels of PUFA present in the mature ovaries and testes of the wild prawn (Middleditch et al. 1979).

Other reports have also noted the importance of lecithin and phospholipid components in crustacean diets. Lecithin denotes a group of fatty substances, mainly phosphatides, that occurs throughout nature as an essential component of living organisms. Many aquatic dietary formulations include lecithin as an ingredient to provide the phospholipids needed by various species and to impart proper physical characteristics to the diet. These range from enhancement of growth, fat and cholesterol digestibility, improved vitamin A and carotene absorption, to retardation of leaching of water- soluble components in the pelleted diet.

This study was conducted to determine the significance of polyunsaturated fatty acids and phospholipids on the reproductive performance, survival and larval quality of ablated pond-reared *Penaeus monodon* broodstock with the use of three practical diets.

Materials and Methods

Three practical diets (A, B and C) were formulated to contain the same basal components (Table 1). Protein and lipid sources consisted of combinations of squid meal, prawn head meal and fishmeal (Peruvian) supplemented with additional sources of lipid; i.e., cod-liver oil (Diet A), soybean lecithin (Diet C) and their 1:1 combination (Diet B). An all-natural diet (squid and marine annelids) served as control.

All the formulated and natural diets were analyzed for proximate chemical composition (Table 2) and constituent fatty acids (Table 3). Lecithin used in this study contained 63% phosphatides mainly as phosphatidyl choline. Although lecithin in the diets was not analyzed, some of the dietary components are sources of lecithin.

Experimental broodstock, eight months old from spawning, weighing 60-128 g (females) and 49-98 g (males) fed on natural food, were obtained from a private fishpond at Villa, Iloilo, Philippines. The animals were stocked in holding tanks for disinfection with 50 ppm formaldehyde (A.R.) for an hour prior to the experiment.

Four flowthrough maturation tanks (4 m diameter, 1.25 m deep) filled to contain 12 m^3 of water were each stocked with 50 broodstock: 22 males and 28 females. Broodstock were acclimated to the diets for 21 days prior to unilateral ablation of females. Feeding consisted of frozen squid 5 times a week and marine annelids twice a week in the morning and formulated diets in the afternoon except for the control which was on all-natural food diet. Daily feeding rates were approximately 5% of the total biomass for formulated diets and 10% for natural diet adjusted for mortalities. Water temperature ranged from 26-31^{o}C and seawater salinity 30-32.5 ppt, measured at 8 to 9 a.m. thrice weekly.

Ovarian development was monitored with strong illumination from underwater flashlight against the dorsal abdominal region of the prawn. Females that reached Stage III ovaries were transferred to 300-l tanks for spawning. The stages adopted were patterned after those reported by Teshima and Kanazawa (1983b). Total number of eggs and nauplii were estimated from three 250-ml aliquot samples taken from spawning tank. The proportion of developed and undeveloped eggs was determined by microscopic examination (Primavera and Posadas 1981). Nauplii from each treatment were reared to zoea stage.

Broodstock performance was evaluated in terms of total number of spawnings, nature of spawning (complete or partial), eggs and nauplii production, hatch rate of eggs and larval quality, measured by percentage metamorphosis to zoea stage.

Total lipid was extracted by the method of Bligh and Dyer (1959) as modified by Kates (1972). Lipid weight was determined gravimetrically and expressed as percent total lipid.

Fatty acid composition was determined by gas-liquid chromatography on a Shimadzu GC-4C PTF gas chromatograph equipped with FID detector and fitted with a 10% diethylene glycol succinate (DEGS) column. Fatty acid methyl esters (FAME) were identified and quantified with an electronic integrator (Hewlett Packard 3390 A). Authentic standards and literature values for published oils (Ackman and Burgher 1965) were used for identification of FAME. Results are presented as FAME weight percent of the total lipid.

Results

Diets A, B and C were found to be similar in fat content, 12.08-12.15%, while protein levels were 52.80-56.49% (Table 2). The diets differed in fatty acid patterns particularly in the amounts of $w3$ and $w6$ polyunsaturated fatty acids (Table 3). Diet A had higher long-chain C_{20} and C_{22} PUFA and $w3/w6$ ratio while Diet C contained more of C_{18} PUFA with low $w3/w6$ ratio.

The relative effects of the diets on the reproductive performance of pond-source *P. monodon* broodstock is shown in Table 4.

Broodstock response in terms of total number and nature of spawnings was best in Diet A with 35 spawnings (79.41% complete) followed by Diet C with 29 spawnings (72.41% complete). High proportion of complete spawnings with females fed Diet A indicates minimal stress in this diet. Although the average number of eggs per spawning was smaller, the average number of nauplii was greater for Diet A than Diet C resulting in higher hatching rate. Diet B gave the poorest overall response among the formulated diets but was better than the control. There were only 15 spawnings in those fed the control diet compared to 20-35 in formulated diets (Table 4) and total egg and nauplii produced were also low. Although maturation rates were similar in all treatments, most of the Stage II and Stage III females fed the control diet resorbed their ovaries and failed to spawn. Survival rates of broodstock over the 120-day culture period was also lowest in the control.

Many of the spawned eggs were unfertilized, suggesting lack of mating and spermatophore deposition or unsuccessful mating due to immature sperms. Nevertheless, fertilized eggs produced good quality larvae. Spawns from all dietary treatments successfully metamorphosed to the zoea stage. The interval between ablation and spawning was 21-32 days; 32 days with Diet A, 21 days with Diet B, 28 days with Diet C and 21 days with the control (Fig. 1). However, spawning frequencies were more consistent in Diets A and C with higher percentage of fertilized eggs. Highest survival rate was 100% for males given Diet C and 60.71% for females fed Diet A. Lowest survival rates were observed in animals fed the control diet.

Discussion

Overall broodstock response was best with Diet A. Cod-liver oil as a dietary component is important as an additional source of C_{20} and C_{22} long-chain polyunsaturated fatty acids. The largest number of spawnings and hatch rates attained with this diet indicate a positive effect of PUFA on the reproduction of *P. monodon*.

Several investigators have reported on the significance of lipids and polyunsaturated fatty acids (PUFA) in maturation diets for broodstock. Middleditch et al. (1979) reported that ovarian maturation and spawning of *P. setiferus* was achieved when the diets were supplemented with lipids rich in C_{20} and C_{22} polyunsaturated fatty acids. The quality and quantity of lipids in the diet have been found essential for the maturation of *P. japonicus* (Teshima and Kanazawa 1983a). Shimma et al. (1977) also found that the hatch rate of eggs from carp fed formulated diets was greatly reduced when the $22:6\omega3$ content of the egg lipid was less than 10%. A recent study on tissue lipid content and fatty acid composition of both unablated and ablated *P. monodon* indicated that $20:4\omega6$, $20:5\omega3$ and $22:6\omega3$ fatty acids were abundant in the mature ovaries of the prawn and were reflected in the spawned egg (Millamena et al. 1985b). The importance of PUFA as a dietary component during the larval development of *P. monodon* was also noted. Prawns generally have demonstrated limited ability to synthesize long-chain PUFA, thus these essential fatty acids have to be provided in their diet.

A variety of reports have noted the importance and essentiality of lecithin and phospholipids in crustaceans diets. Lee and Puppione (1978) noted that phospholipid is the principal form of lipid in the crustacean hemolymph. Teshima and Kanazawa (1979) further suggested that phospholipids are required for lipid transport in *P. japonicus* and are a very important component in their diet. D'Abramo et al. (1981) also noted the importance of phosphatidyl choline as a component of lipoprotein complex that transfer cholesterol from the hepatopancreas to the hemolymph. Likewise, soy lecithin and phospholipids incorporated in the diet gave high survival in juvenile lobsters (Conklin et al. 1980) and in *P. japonicus* (Deshimaru 1981).

The effect of lecithin in maturation diets for *P. monodon* has still to be elucidated. In this study, lecithin as an additional lipid source improved fecundity and survival rates of male broodstock but produced lower hatching rates compared to cod-liver oil. Low hatching obtained with Diet C may be due to lower C_{20} nd C_{22} PUFA in the lecithin diet although this is high in C_{18} PUFA. Excessively high levels of $18:2\omega6$ and $18:3\omega6$ fatty acid in the diet was also reported to have toxic effect on the prawn.

Lack of fertilization may be due to factors such as sex ratio (four females: three males) and age of broodstock; these may explain partly why some females failed to mate or mated but spawned unfertilized eggs. Diet quality may also have a significant effect on egg development. Percentage of unhatched eggs in females fed Diet A was low (31.5%) compared to the rest of the treatments (55.2% to 66.7%).

There is apparent need for formulated diets to supplement natural food for pond-grown broodstock. The slow response of pond broodstock to ablation compared to wild may be traced back to their dietary history. Pond-reared broodstock are mainly dependent on available food in the pond for their nutrition while wild broodstock are exposed to a varied and more complete diet.

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Table 1. Percentage composition of experimental diets for *P. monodon* broodstock.

Ingredients	Diet A	Diet B	Diet C
Squid meal	30.00	30.00	30.00
Shrimp head meal	20.00	20.00	20.00
Fish meal	20.00	20.00	20.00
Wheat flour	5.50	5.50	5.50
Gulaman	4.00	4.00	4.00
Vitamin mix	2.70	2.70	2.70
Mineral mix	6.00	6.00	6.00
Rice bran	5.30	5.30	5.30
Cod liver oil	6.00	3.00	0.00
Lecithin	0.00	3.00	6.00
Cholesterol	0.50	0.50	0.50
BHT	0.03	0.03	0.03

Table 2. Proximate chemical composition (%) of broodstock diets.

	Percent moisture	Crude protein	Crude fat	Crude fiber	NFE	ASH
Diet A	3.34	52.80	12.14	3.80	13.39	17.87
Diet B	2.95	53.55	12.08	3.83	12.10	18.44
Diet C	6.17	56.49	12.15	4.92	9.94	16.50

Table 3. Fatty acid profiles of formulated diets for *P. monodon* broodstock.

Fatty acid	Diet A	Diet B	Diet C
14:0	0.04	0.14	0.44
14:1	1.17	0.98	–
15:0	0.13	0.14	0.08
16:1w7	7.48	17.64	16.20
16:2	6.38	–	5.90
17:0	6.36	6.35	–
18:1w9	14.28	16.02	14.93
18:2w6	7.16	13.21	20.51
18:3w6	3.82	4.38	9.27
18:3w3	5.20	4.13	2.92
20:1	12.65	10.03	4.88
20:4w6	6.48	5.10	5.12
20:5w3	7.28	6.81	5.56
22:1	11.78	8.35	5.35
22:4w3	–	–	1.22
22:5w6	2.05	1.69	1.54
22:5w3	1.60	1.00	0.93
22:6w3	6.15	5.09	4.93
w3/w6	1.03	0.70	0.41

Table 4. Spawning, fecundity, hatching rate and survival of ablated pond-source *P. monodon*.

Parameter	Diet A	Diet B	Diet C	Control
Number of spawnings[1]	35^{a}	20^{bc}	29^{ab}	15^{c}
With hatching	24 (68.5%)	8 (40%)	13 (44.8%)	5 (33.3%)
Without hatching	11 (31.5%)	12 (60%)	16 (55.2%)	10 (66.7%)
Nature of spawning[2]				
PS	20.59%	30.00%	27.59%	26.67%
CS	79.41%	70.00%	72.41%	73.34%
Total no. of eggs	6,967,000	3,853,000	6,410,000	2,724,000
Total no. of nauplii	2,555,000	1,068,000	1,252,000	609,000
Percent hatching rate	36.67%	27.27%	19.53%	22.36%
Average no. of eggs/sp.	204,912	192,650	221,034	181,600
Average no. of nauplii/sp.	75,147	53,400	43,172	40,600
Survival rate (%)				
Males	85.71%	85.71%	100.00%	47.62%
Females	60.71%	57.14%	57.14%	42.86%

[1] Treatment means with the same superscripts are not significantly different at $P < 0.05$.

[2] Partial spawning.

Complete spawning.

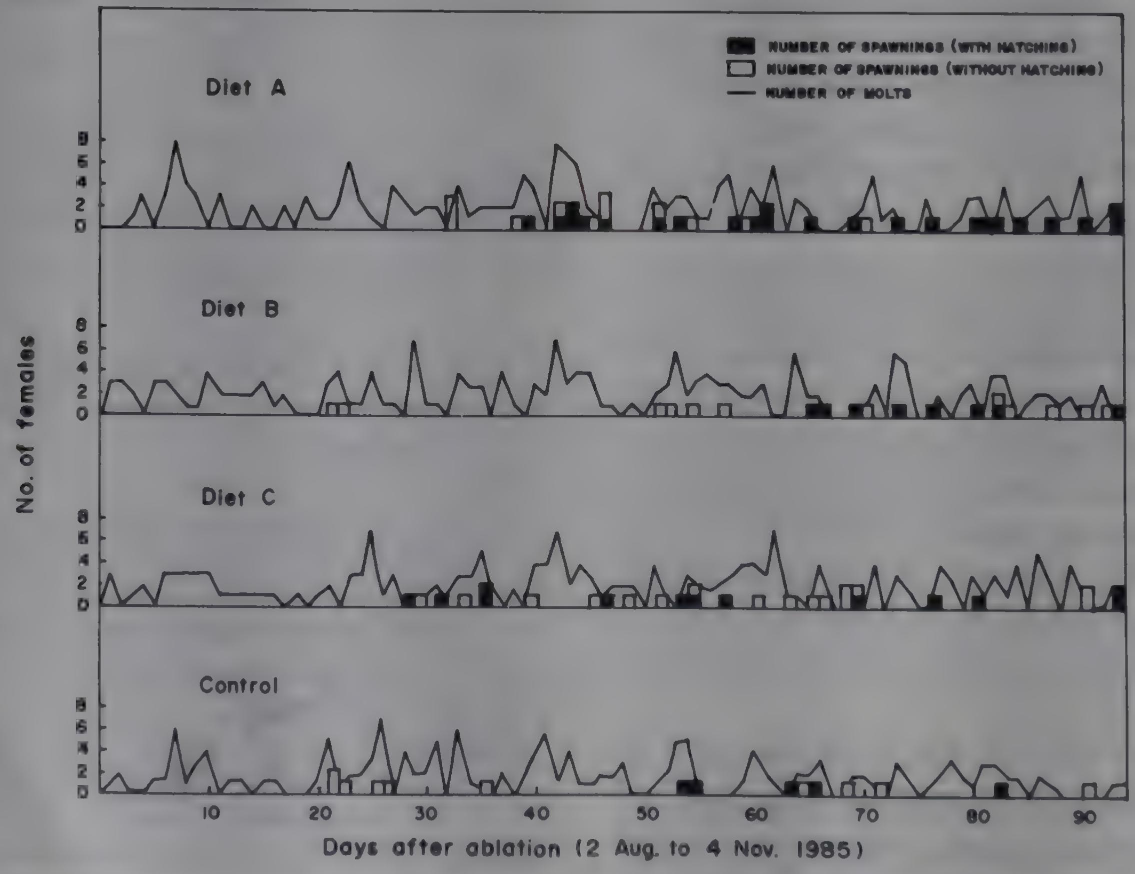

Fig. 1. Number of molts and spawnings of ablated pond-reared *P. monodon* fed different diets.

Enriched Conventional Feed for Indian Major Carps

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Abstract

The conventional fish feed comprising 1:1 groundnut oil cake and rice bran by weight was enriched with an aquatic weed *(Salvinia* sp.*)* powder, bloodmeal and sugar wastes and two test diets were prepared with a protein level of 28-29%. Diet A was composed of bloodmeal, ricebran, and groundnut oil cake (1:3:6) while Diet B of groundnut oil cake, *Salvinia* powder and sugar wastes (8:1:1). All the experimental diets were fortified with vitamins and minerals. The two diets were tested against the conventional feed in a laboratory trial run of 60 days on fry of *Labeo rohita* (Ham.). Significantly improved growth ($P < 0.01$) was observed from the feed with bloodmeal compared to the other two diets.

Introduction

Supplementary feed constitutes a major input in aquaculture. Since animal protein sources are expensive, the need for reasonable substitutes is paramount. This study aimed to utilize ingredients from locally available, cheap alternative plant and animal sources especially those unsuitable for direct human consumption. To develop acceptable diets from such sources, bloodmeal was tried as a partial or complete substitute for fishmeal and *Salvinia* sp., an aquatic weed, was fortified with sugar wastes or rice bran.

Bloodmeal was produced from clean, fresh goat and sheep blood which did not include any extraneous material such as hair, stomach belchings and urine, except in such traces that unavoidably occur in the nearby slaughterhouse. A large proportion of the moisture was removed by condensation through cooking to a semi-solid state. The semi-solid mass was then transferred to a hot air oven and dried at 60°C for 24 hours.

Materials and Method

The experiments were conducted in November 1984-January 1985. Acclimatized rohu *(Labeo rohita)* fry were randomly selected and introduced into plastic pools 59.5-cm high and 90-cm diameter filled with 150 l of water after ensuring complete evacuation of food from their guts by starving the fishes for 24 hours. Each treatment had three replicates with ten fry in each pool. While the temperature varied from 29.8 to 34.0°C, the oxygen level of water was maintained at about 9 ppm by using aerators. The fish were fed once a day at 10% body weight and the water in the plastic pools was changed every other day.

The test diets were formulated after ascertaining moisture (loss at 100°C for 12 hours), protein (Microkjeldahl N x 6.25) (AOAC 1970), fat (petroleum ether extraction by Soxtec extraction 1040 unit), ash (residue after heating at 600°C for 6 hours) and carbohydrate (Benedict's method) (Oser 1965). The two diets were isonitrogenous (28-29%). Diet A was made up of bloodmeal, rice bran and groundnut oil cake (1:3:6), while diet B had groundnut oil cake, *Salvinia* powder and sugar wastes (8:1:1). The control, Diet C, was a 1:1 mixture of groundnut oil cake and rice bran (20.36% protein). All the diets were fortified with vitamins and minerals. The proximate compositions of the prepared diets were analyzed and presented in Table 1.

Results and Discussion

The results of the feeding trial are presented in Table 2. Fish fed Diet A exhibited the maximum average weight gain (0.8 g) and specific growth rate (1.75%). Two-way analysis of variance (Table 3) indicated significant growth differences between the fish fed different diets on 10, 20, 30, 40, 50 and 60 days ($P < 0.01$).

Although both Diets A and B were isonitrogenous, the better performance of Diet A may be due to easy digestibility of bloodmeal (FAO 1983) and availability of all essential amino acids (Dupree and Halver 1970) resulting in higher protein deposition in flesh of fry (23.27%). The superiority of bloodmeal as a feed component has also been reported for channel catfish (Reece et al. 1975) and salmon (Fowler and Banks as reported by FAO 1983).

The absence of essential amino acids, the lower digestibility of plant protein and the high percentage of carbohydrates in Diet B may be the causes for its poor performance. Further, as a large proportion of carbohydrate reduces digestibility (Maynard 1947), the protein deposition was also low (21.82%) in the flesh of rohu fry fed this diet.

Results indicate the prospects of inexpensive bloodmeal as a partial or complete substitute for costly fishmeal in commercial rations for the Indian major carps.

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Table 1. Proximate composition of diets* fed to rohu fry.

Feeds	Moisture %	Protein %	Fat %	Carbohydrate %	Ash %	Crude fiber %
Conventional feed mixture of ground nut, oil cake and rice bran. Diet C	10.2	20.36	10.97	35.25	21.06	12.36
Mixed feed of ground nut, oil cake, aquatic weed *(Salvinia)* and sugar wastes. Diet B	9.6	29.09	20.07	34.68	7.3	8.86
Mixed feed of ground nut, oil cake, rice bran and blood meal. Diet A	9.8	28.00	12.10	32.83	9.8	17.27

*Moisture content expressed as percentage of fresh diet. Protein, fat, carbohydrate as percentage of dry weight of diet.

Table 2. Specific growth rate of rohu fry fed test diets.

Diets	Average initial live weight, S.D. and range (g)	Average final live weight, S.D. and range (g)	Average live weight gain (g)	Specific growth rate (% wt/day)
Conventional feed mixture of ground nut oil cake and rice bran.	0.48 ± 0.10	0.92 ± 0.09	0.44	1.08
Diet C	(0.38 – 0.58)	(0.83 – 1.0)		
Mixed feed of ground nut oil cake, aquatic weed *(Salvinia)* and the sugar wastes.	0.57 ± 0.16	1.02 ± 0.08	0.45	0.97
Diet B	(0.41 – 0.72)	(0.96 – 1.10)		
Mixed feed of ground nut oil cake, rice bran and bloodmeal.	0.43 ± 0.16	1.23 ± 0.21	0.80	1.76
Diet A	(0.26 – 0.54)	(1.00 – 1.40)		

Table 3. Two-way analysis of variance of average live weight gains of rohu fry fed test diets.

Source of variation	Degree of freedom	Sum of squares	Mean square	Variance ratio (F)	Probability values
Column (feed)	2	0.1723	0.08615	9.63	P <0.01*
Row (days)	5	0.6857	0.13714	15.32	P <0.01*
Error	10	0.0895	0.00895		
Total	17	0.9475			

*Highly significant.

Stocking Density and Diet of *Oreochromis niloticus* in Cages in Manmade Lakes in Sri Lanka (II)

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Abstract

Oreochromis niloticus were cultured in cages (rearing volume 5 m^3) in the Udawalawe reservoir in the Sabaragamuwa Province, Sri Lanka, to evaluate the optimum stocking density to maximize fish production. *O. niloticus* fingerlings of 22-30 g were stocked at four stocking densities (400, 600, 1,000 and 1,200 fish/m^3) and were fed two pelletized diets with crude protein levels of 17% and 20%. The fish were fed at 3% of body weight six times daily. The trial period lasted 150 days in the first instance. After 150 days, the fish in the 400 fish/m^3 stocking density cage were spread out to eight cages at stocking density of 25 fish/m^3 and cultured further for 30 days. The trials were carried out in three replicates. All stocking densities registered no significant difference in the live weight gain (LWG) per fish fed the two diets at the end of 60 days ($P > 0.05$). After 150 days, no significant difference in the LWG values of the fish was observed in all stocking densities for a particular diet ($P > 0.05$). However, the LWG values of fish at 400 and 600 fish/m^3 groups differed significantly from those at 1,000 and 1,200 fish/m^3 for the two diets ($P < 0.05$). After thinning out the fish to 25 fish/m^3, there was an increase of 40% body weight per fish after 30 days of culture, decreasing the feed conversion ratio. Although the final biomass increased with increase in stocking density, the net weight gain per 1,000 fish decreased, indicating that the best stocking density over the range tested was between 400 and 600 fish/m^3.

Introduction

The importance of cages and pens for culturing suitable fish is well recognized throughout the world and well documented (Coche 1982). Extensive work on tropical cage culture of *Oreochromis niloticus* has been carried out in the Philippines. Intensive cage culture of *O. niloticus* at varying stocking densities with different protein diets have been described (Coche 1977; Campbell 1978; Guerrero 1979). Campbell (1985) described the technique of producing large numbers of calibrated fish for rearing in commercial cages. Preliminary work carried out in Sri Lanka on *O. niloticus* in cages was described by Galapitage (1982). Exhaustive work on intensive cage culture of *O. niloticus* (Wannigama and Weerakoon 1982; Wannigama et al. 1985; Muthukumarana and Weerakon 1985) showed that the growth of *O. niloticus* was not significantly influenced by feeds with protein contents varying between 19 and 29% at stocking densities of 400, 600 and 800 fish/m^3. The present work continues the evaluation of the optimum stocking density for maximizing the production of marketable *O. niloticus* from cages.

Materials and Methods

Trials with *O. niloticus* were carried out in Udawalawe reservoir in the Sabaragamuwa province in the intermediate dry zone of Sri Lanka which has a catchment area of 1,162 km^2 (De Silva et al. 1984). At full spill level (FSL) the reservoir has an area of 3,374 ha corresponding to a volume of 2.55×10^{12} m^3 and a mean water depth of 78.3 m. The dead storage capacity is 1.66×10^{11} m^3. The full supply level is at 87.5 m. Plankton densities at different depths are given in Tables 1a and 1b.

The cage design and construction used are amply described by Wannigama and Weerakoon (1982). The frames were made of bamboo and the net cage of kuralon or nylon netting, mesh and ply 1-2 cm and 6-9, respectively. The best cage design which proved to be efficient and withstood adverse weather conditions consisted of a floating bamboo square frame with platforms as walkways, the net cage suspended from stilts attached to the four corners of the bamboo frame. The bamboo cage frame was 3 x 3 m and the effective volume of the net cage was 5 m^3. The four stocking densities were 400, 600, 1,000 and 1,200 fish/m^3. Fish at each stocking density were fed two diets, F_1 and F_3, with protein contents of 17% and 20%, respectively. The feed formulations (Table 2) were fed 3% by body weight, in pelleted form broadcast by hand every two hours six times daily. There was no loss of feed as fish fed on the pellets immediately upon introduction into the cage. Sampling of fish was carried out every fifth week, and a random sample of 10% of the population was measured for total length and weight. Other parameters, such as dissolved oxygen, temperature and conductivity were measured fortnightly. The trial lasted 150 days after which the fish in the 400 fish/m^3 cage were thinned out to six cages at 25 fish/m^3. After thinning, fish were reared for another 30 days and the fish in separate cages fed both diets F_1 and F_3.

Results

From 400 to 600 fish/m^3 stocking densities, the variation between the live weight gain (LWG %) values of diets F_1 and F_3 for a particular stocking density, as well as between stocking densities for a particular diet was not significant ($P > 0.05$) as shown in Table 3. The same was true for 1,000 and 1,200 fish/m^3 stocking densities with diets F_1 and F_3. However, when LWG value at 400 fish/m^3 was compared with those at 1,000 and 1,200 fish/m^3 there was a significant difference ($P < 0.05$) for diets F_1 and F_3.

An analysis of variance was carried out with final mean weights of fish fed diets F_1 and F_3 between 400 and 1,200 fish/m^3 stocking densities based on relevant data (Table 4) at the end of the culture period (150 days). The difference in the final mean weights between stocking densities for a particular diet (F_1 and F_3) was significant ($P < 0.05$).

The growth of *O. niloticus* in 60 days at the four stocking densities was almost similar (Fig. 1), the difference being due to the variation in the initial weight of 23.0-30.7 g. The computed F values with LWG values at the end of 60 days, showed that there was no significant difference in the LWG values at each stocking density for diet F_1 or F_3 ($P > 0.05$). The growth of *O. niloticus* at 400 fish/m^3 fed diets F_1 and F_3 after thinning to 25 fish/m^3 stocking density (Fig. 2) was significantly faster 30 days after thinning, with a weight gain of nearly 40%.

The final total fish weight (B_f) at the end of the culture period shows that as the stocking density increased, the B_f increased, but the B_f at 1,200 fish/m^3 stocking density decreased well below the B_f for 1,000 fish/m^3. Although the B_f increases with higher density, the total weight gain per 1,000 fish (GN/1,000) tended to decrease, with higher density, implying that 600 fish/m^3 is the maximum stocking density under conditions described in this paper.

The feed conversion rates (FCR) were 3.1-6.7 and were comparatively higher for diet F_1 at all stocking densities (Table 4). At 400 and 600 fish/m^3, the FCR for diet F_1, was higher than for diet F_3, which was significant ($P < 0.05$). Similarly, the FCR values for diets F_1 and F_3 at 400 and 600 fish/m^3 which were 4.1 and 3.2 and 4.8 and 3.1, respectively, were significantly different when compared with the FCR values for the same diets at 1,000 and 1,200 fish/m^3 ($P < 0.05$). The FCR values in parenthesis represent the FCR recorded after thinning in 150 days and the fish cultured for another 30 days. Thinning followed by a 30-day culture period, effectively decreased the FCR at 400 fish/m^3 for both diets F_1 and F_3, from 4.2 to 2.7 and from 3.2 to 2.5, respectively.

Discussion

Intensive cage culture of *O. niloticus* was reported by Coche (1977), Campbell (1978) and Guerrero (1979). In most of the cases, the type of feed presented, the initial weight per fish, the size of cages and the culture period differ from those of these tests. Wannigama et al. (1985) and Muthukumarana and Weerakoon (1985), showed tht diets with protein contents varying between 19 and 29% did not produce a significant difference in the growth rate of *O. niloticus*, although a diet with protein content of 25% exhibited a comparatively better FCR for all stocking densities tested (400, 600 and 800 fish/m^3).

In this paper, data show that diet F_3, with a protein content of 20% produced the best FCR values at 400 and 600 fish/m^3 when subjected to a single stage culture system of 150 days without thinning. Although the B_f value was highest at a stocking density of 1,000 fish/m^3, the GN/1,000 fish decreased. This, coupled with the fact that the B_f value for 1,200 fish/m^3 decreased below the B_f values for 1,000 fish/m^3, shows that the maximum holding capacity for the 5-m^3 cage, after which the growth rate of *O. niloticus* tended to decrease, was 600 fish/m^3. Therefore, we assume that the best stocking density over the range tested was between 400 and 600 fish/m^3.

Significant is the similarity in fish growth at all stocking densities tested within the first 60 days (Fig. 2). It can be implied that in intensive cage culture, *O. niloticus* could be initially stocked at 1,200 fish/m^3 for 60 days, after which thinning out of fish to the desired stocking density for further culture to marketable size could be carried out. This also reduces the number of cages for culture operations to a minimum.

After thinning at the end of 150 days, the increase in weight after 30 days of culture could be attributed to availability of space. The decrease in the FCR to 2.7 and 2.5 for diets F_1 and F_3, respectively, at 400 fish/m^3 as a result of this, is also most significant as a higher mean weight per fish at harvest was achieved in 180 days with a lower FCR. It is also evident that a FCR similar to that obtained by Muthukumarana and Weerakon (1985) with a diet of 25% of crude protein, could be achieved by a two-stage culture system (150 + 30 days), with a diet containing 17 or 20% crude protein at 400 fish/m^3. In addition the final mean weight at harvest was higher, 158 and 175 g for the two diets, respectively. The B_f also increased from 243.7 to 333 kg (from 48.7 to 66.6 kg/m^3) with diet F_3 and from 174.2 to 281 kg (from 43.8 to 56.2 kg/m^3) with diet F_1. This was comparable to the work carried out by Coche (1977) with *O. niloticus* at 349 fish/m^3, fed a diet containing crude protein of 24.7%.

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Table 1a. Phytoplankton density at various depths in the reservoir, Station II, 1984

Depth m	August (unit/l)	October (unit/l)	December (unit/l)	February (unit/l)
Surface	(1.82×10^4)	(2.01×10^4)	(8.4×10^3)	(8.12×10^3)
1	(2.28×10^4)	(2.3×10^4)	(9.88×10^3)	(1.42×10^4)
2	(2.2×10^4)	(2.49×10^4)	(9.62×10^3)	(9.66×10^3)
3	(2.42×10^4)	(2.22×10^4)	(9.8×10^3)	(9.1×10^3)
4	(1.99×10^4)	(1.66×10^4)	(6.92×10^3)	(8.71×10^3)
5	(1.87×10^4)	(1.14×10^4)	(6.88×10^3)	(3.46×10^3)
Ave. no. of phytoplankton/l*	(2.1×10^4)	(1.97×10^4)	(8.67×10^3)	(8.88×10^3)

***Scenedesmus* sp., *Pediastrum* sp. and *Microcytis* sp. are permanent species.

Table 1b. Zooplankton density at various depths in the reservoir, Station II, 1984.

Depth m	August (no/l)	October (no/l)	December (no/l)	February (no/l)
Surface	460	800	128	44
1	252	800	60	28
2	320	1,500	30	72
3	240	880	96	106
4	224	1,400	72	58
5	67	816	68	56
Ave. density no./l*	259	1,032	75	60

*Rotifers and cladocerans are most dominant.

Table 2. Composition of diets with dietary crude protein levels.

Ingredients	Diets (%) F_1	F_3
Fishmeal	05	10
Chick mash	15	45
Rice bran	77	42
Sharkliver oil	03	03
Overall crude protein level	17.2	20.0

Table 3. Percentage live weight gain (LWG) of *O. niloticus* fed two dietary crude protein levels at four stocking densities for five months.

Diet	Protein level	% LWG 400/m^3	600/m^3	1,000/m^3	1,200/m^3
F_1	17.2	238	200	174	123
F_3	20.0	296	337	152	132

Table 4. Final biomass (B_fW), net gain/1,000 fish (GN/1,000) and feed conversion rates (Fd/GN) for various dietary crude protein levels at four stocking densities of *O. niloticus*.

Dt/CP	B_iW	B_fW	Surv. %	M_fW/ fish/g	GN/ kg/cage	GN/1,000 kg	Fd/Gn
2,000 fish/cage ($400/m^3$)							
17%	58.80	174.20 (281)	88.20	98.8 (168)	115.40 (222.2)	69.35 (129.9)	4.10 (2.7)
20%	61.40	243.70 (332.7)	94.70	121.6 (176)	182.30 (271.3)	97.96 (144.9)	3.20 (2.5)
3,000 fish/cage ($600/m^3$)							
17%	89.70	230.10	91.80	83.5	140.40	53.65	4.80
20%	89.40	330.00	97.70	110.0	240.60	82.78	3.10
5,000 fish/cage ($1,000/m^3$)							
17%	147.00	366.60	98.70	74.3	219.60	44.88	5.70
20%	163.00	382.10	85.40	89.4	219.10	56.88	4.90
6,000 fish/cage ($1,200/m^3$)							
17%	148.20	310.90	93.70	55.3	162.7	30.60	5.90
20%	155.40	346.00	95.80	60.2	190.60	34.29	6.70

Value in parentheses for FBW, GN/cage, M_fW and GN/1,000 were obtained after thinning out to 25 fish/m^3 after the fifth month and 30 days extended culture.

B_iW = initial total fish weight; B_fW = final total fish weight; GN/cage = net gain/cage; GN/1,000 = net gain/1,000 fish; Fd/Gn = feed conversion ratio; M_fW = mean weight/fish at harvest.

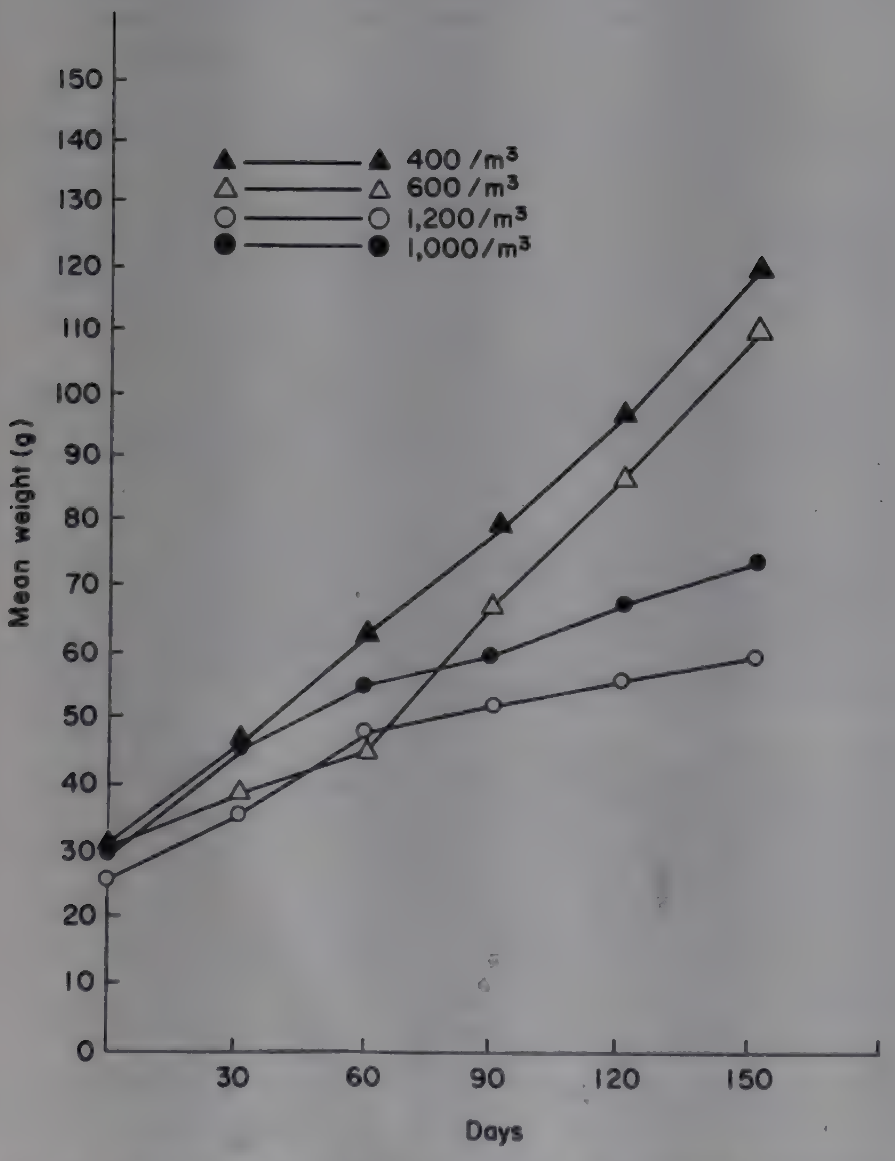

Fig. 1. Growth rate of *O. niloticus* at four stocking densities fed diet F_3 (20%).

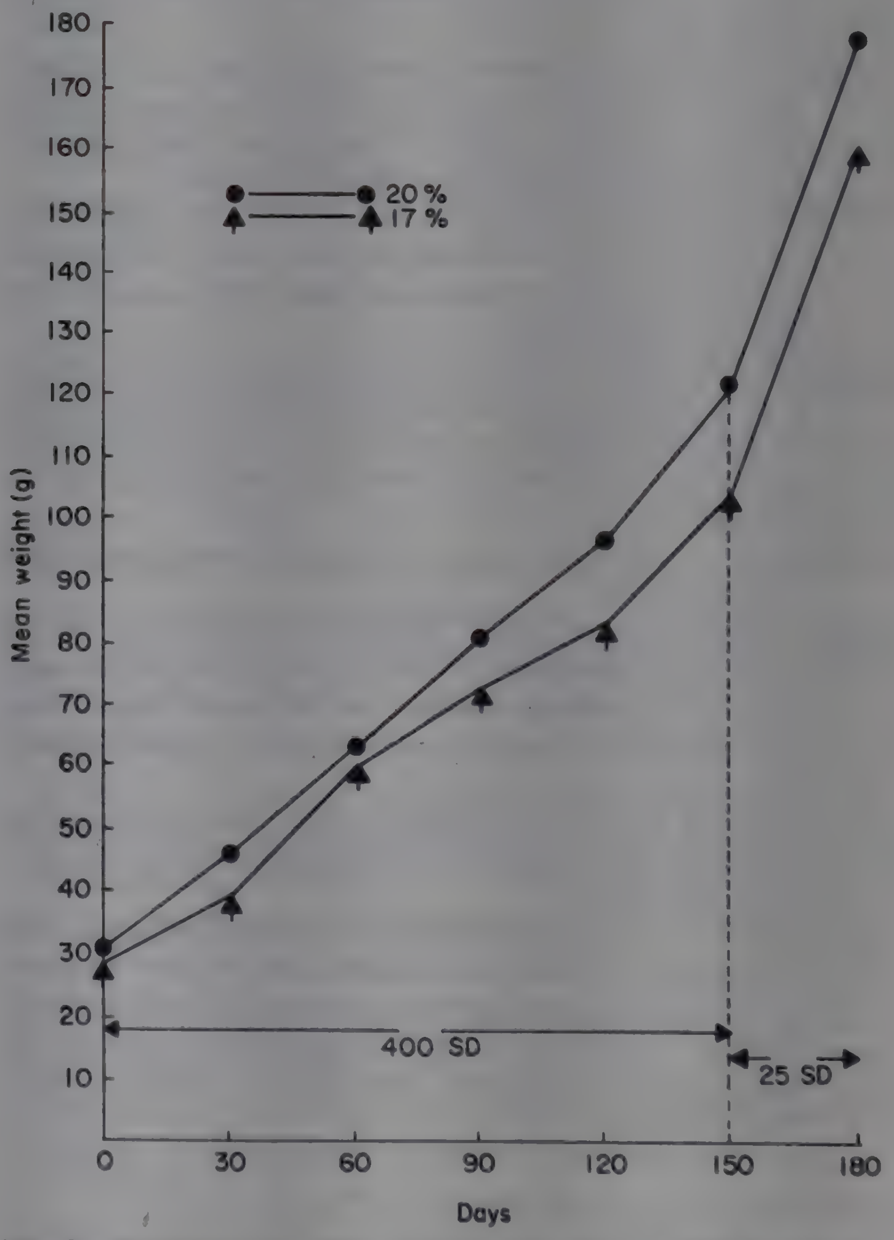

Fig. 2. Growth rate of *O. niloticus* fed diets F_1 (17%) and F_3 (20%) at stocking density of 400 fish/m^3, first 150 days and at 25 fish/m^3 after a 30-day extension.

Growth Response of Four Species of Carps to Different Protein Sources in Pelleted Feeds

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Abstract

Experiments were conducted in duplicate in earthen ponds of 200 m^2 each for a period of 112 days. Four species of major carps -- catla, rohu, common carp and silver carp were stocked at 7,000/ha in the ratio of 0.5:1:1:1, respectively. Three feeds -- silkworm fecal matter based diet (pellet SF), slaughter house waste based diet (pellet SH) and fishmeal based diet (pellet FM) were fed at 5% of the body weight of the fish for the first 28 days and later at 2.5% of the body weight. All the three feeds were formulated in such a way that the protein content of each feed was around 30%.

The growth of catla was found to be superior on pellet SH, followed by pellet FM and pellet SF. The growth of rohu was found to be superior on pellet SF, while it was almost equal on pellets SH and FM. Silver carp responded better to pellet SH than to pellets SF and FM. Common carp was found to grow better on pellet SH than on pellets SF and FM. Net estimated fish production obtained from pellet SH was 2,097.80 kg/ha/year as against the production from pellet SF (1,684.18 kg/ha/year) and pellet FM (1,148.40 kg/ha/year). Conversion ratio was found to be the best (1.67) with pellet SH, followed by 1.93 for pellet FM and 2.03 for pellet SF. The cost of production of fish was maximum with pellet FM (Rs. 3.70/kg) as compared to pellet SH (Rs. 2.52/kg) and pellet SF (Rs. 1.97/kg). This study indicates that it is possible to successfully replace fishmeal with cheaper slaughter house waste or silkworm fecal matter.

Introduction

The importance of supplementary feeding has been well realized in aquaculture in India and has stimulated intensive research on the formulation of cheaper feeds. In this investigation, an attempt was made to develop two cheaper nutritive diets with slaughter house waste and silkworm fecal matter as the principal sources of protein. These ingredients are rich in protein and are easily available at low cost. The diets developed were tested by comparing their performance with a standard fishmeal-based diet fed to carps.

Materials and Methods

The experiments were conducted over a period of 112 days. The three feeds used in the experiment were designated as pellet SH (slaughter house waste-based diet), pellet SF (silkworm fecal matter-based diet) and pellet FM (fishmeal-based diet).

The crude protein, ash and moisture contents were determined following AOAC (1975) procedures. Total lipid content was measured by the method of Bligh and Dyer (1959). Crude fiber analysis was carried out following the method of Lees (1975), while nitrogen free extract was estimated by the difference method (Hastings 1976).

Each treatment was duplicated in uniform-sized earthen ponds of 200 m^2 each. A fortnight prior to the start of the experiment, the ponds were drained and dried, uniformly limed at 200 kg/ha and their soil beds raked. They were then filled with water to 1-m height and this level was maintained throughout. Each pond was stocked with four species of carps: catla *(Catla catla)*, rohu *(Labeo rohita)*, silver carp *(Hypophthalmichthys molitrix)* and common carp *(Cyprinus carpio)* in the ratio 0.5:1:1:1 at a total stocking density of 7,000 fingerlings/ha.

Fish growth was recorded fortnightly and the quantum of feed given was determined based on the total weight of fishes. Feeding was done once a day in the early morning at 5% body weight for the first 28 days and at 2.5% for the remaining period.

Water samples were also collected fortnightly and analyzed for pH, oxygen, carbon dioxide and total alkalinity following APHA (1975) methods. The growth of each species was subjected to two-way analysis of variance (Snedecor and Cochran 1968) to find out the statistical difference in the final mean weight of fishes in the three treatments.

Results and Discussion

Two batches of feeds were prepared during the experiment. The proximate composition of the ingredients and their proportion in the three diets are presented in Table 1 and the proximate composition of the formulated diets in Table 2.

The protein content of fishmeal (61.32%) in the first batch of feeds was slightly higher than that of slaughter house waste (60.59%). But the slaughter house waste in the second batch had very high protein (86.85%) and low lipid content (5.10%). This variation was mainly due to difference in the waste composition, which consisted of inedible portions of the meal, adipose tissue, liver, etc. Silkworm fecal matter contained less protein than the other two principal protein sources (Table 1). However, the protein values recorded for fecal matter in this study are much higher than the values of 12 to 15% reported by Yokoyama (1962) and Jeyachandran and Paulraj (1976) for silkworm litter. The fecal matter was devoid of other wastes like the inedible portion of mulberry leaves. These investigations show that the crude protein content of silkworm fecal matter varies between 20 and 45%, depending on the age and variety of silkworm, storage conditions, variety of mulberry leaves and soil nutrient of the mulberry plot.

The caloric content of the feeds varied, the highest being in pellet SH and the least in pellet SF. Pellet SF had the highest crude fiber content which appears to have been mainly contributed by the silkworm fecal matter. A crude fiber level of 21% is known to reduce the growth of catfish (National Research Council 1977).

The water temperature ranged from 24.5 to 27.5°C over the experimental period. The pH remained alkaline throughout in all the ponds but its value did not exceed 9. The carbon dioxide values varied from nil to 15 ppm in different treatments. The total alkalinity in the three treatments ranged between 114 and 140 ppm. Dissolved oxygen showed fluctuation in all the treatments (Fig. 1).

The growth of catla in terms of weight is presented in Fig. 2. The final weight attained by catla was the highest with pellet SH, followed by pellets FM and SF. The growth in SF treatment was higher up to the 84th day and became faster with pellet SH until the termination of the experiment. A rapid increase in growth was recorded in FM treatment after the 70th day, which ultimately registered better growth only next to pellet SH fed fish. The average daily increments in weight were 1.42 g, 1.10 g and 0.99 g/day in SH, FM and SF treatments, respectively.

Ranade and Kewalramani (1967) reported that catla, being a zooplankton feeder, is better equipped to utilize animal protein. Although diets SH and FM contained animal protein as their principal protein component, the best growth obtained with the former diet could be due to its higher energy content. The sudden spurt in the growth of catla recorded after the 70th day in FM treatment could be attributed to the improved oxygen availability (Fig. 1). Prior to that day, oxygen remained at low levels in this treatment. According to Brown and Gratzek (1980), if the oxygen concentration is less than 4 ppm, fish may survive, but growth is inhibited. The higher lipid content of pellets SH and FM could have contributed to the better growth of catla, sparing protein for growth. In rainbow trout, a lipid level of 5% is known to impair the growth, while a diet containing 10% lipid or more is known to enhance it (Takeuchi et al. 1978).

Rohu recorded the best growth with pellet SF throughout the experimental period. Although the final weights gained in both SH and FM treatments were almost equal, growth remained slow in the latter treatment until the 56th day and impressively picked up thereafter (Fig. 3). The daily increase in weight was also higher in SF treatment (0.65 g/day). Between SH (0.55 g/day) and FM (0.54 g/day) treatments, the difference was negligible.

The best growth of rohu obtained with pellet SF is indicative of the suitability of plant protein for this species. Rohu, being herbivorous (Hora and Pillay 1962), may have different dietary requirements compared to other carps. Anil (1981) recorded the best growth of rohu with water hyacinth leaf powder-based feed against that of fishmeal-based feed which clearly supports the present finding. Further, it is interesting to note from Anil's results that a crude fiber level of 16.62% in the former feed did not have any adverse effect on growth; instead it appears to have helped in the digestibility. Among the three pellets tried in this experiment, pellet SF had the highest crude fiber content. However, in the diets of catfish, a crude fiber level of 21% reduced the growth rate, while a percentage of less than eight resulted in good growth (National Research Council 1977). The desirable level for carps is not clear and appears to vary with the natural food habits of each species. In addition to its advantages, pellet SF also possessed an attractive odor which might have been relished by rohu. According to Kamal (1967), rohu is a taste feeder. Further studies conducted to evaluate the organoleptic qualities of fish fed on the three diets employed in this investigation showed better odor in the flesh of rohu grown on pellet SF compared to those raised on pellet FM (Nandeesha et al., unpublished data).

The growth of silver carp varied highly among the different treatments. It remained superior throughout the period in SH treatment, followed by SF and FM (Fig. 4). The daily increase in weight was the highest in SH (1.81

g/day) and the least in FM (0.66 g/day). Compared to these two treatments, the growth recorded with pellet SF was moderate (1.37 g/day). Although it is very difficult to draw any inference for the slow growth rate of silver carp under FM because of its sensitivity to changing environmental conditions, the low oxygen concentration in pellet FM ponds might have impaired its growth. Further, catla and silver carp being surface feeders, there appears to be an interspecific competition for food between these two species.

The growth of common carp was superior with pellet SF, followed by pellets SH and FM until the 70th day. Later on, pellet SH fed fishes recorded better growth than those in the other two treatments (Fig. 5). Similar to other fishes, the growth of common carp picked up substantially after the 70th day in FM treatment. The daily increment in weight was the highest in SH treatment (1.20 g/day) compared to SF (0.92 g/day) and FM (0.83 g/day).

The better growth of common carp on slaughter house waste based feed and silkworm fecal matter based feed implies their acceptability and utilization by common carp. Cruz and Laudencia (1978) observed good growth of tilapia fed on mulberry leaf meal. Contrary to this, Jeyachandran and Paulraj (1976) observed negative growth of common carp when fed directly with silkworm litter and suspected that it may contain some toxic components like saponin. Nandeesha (1982) also suspected the toxicity of silkworm fecal matter as pond manure and suggested that with proper heat processing it could be utilized as a feed ingredient as it contains a good amount of nitrogen. The fairly good growth of the carps obtained with pellet SF indicates the detoxification of the toxic components or inactivation of anti-nutritional factors that could be present in silkworm fecal matter. The lower growth of common carp with pellet FM could be mainly due to the initial suppression in growth. The results of the statistical analysis showed no significant difference ($P < 0.1$) in the final mean weight gained by common carp in different treatments. This statistical inference was also true to the other three carps in the experiment.

The cost of feed, conversion rate, overall survival and net fish production are presented in Table 3. As the cost of silkworm fecal matter is low, the cost of pellet SF was the lowest. The overall survival of the different species was better in SH (70.00%), followed by SF (69.13%) and FM (62.81%). The net fish production obtained was also the highest in SH. The relative conversion rate was the best with pellet SH (1.67) and only a small variation was noticed between pellet SF (2.03) and pellet FM (1.95). As the relative conversion rate does not indicate the actual feed impact, the variations could also be due to natural food availability. As the cost of pellet FM is high, the cost of fish production was maximum in that treatment (RS. 3.70/kg), while it was lowest with pellet SF (Rs. 1.97/kg).

Results indicate the possibility of replacing fishmeal with slaughter house waste or silkworm fecal matter. Although the poor growth rate of fish fed with pellet FM has been partly attributed to poor water quality, the better growth rate of carps with pellets SH and SF clearly indicates that their principal protein sources are equally good as fishmeal protein. Balanced diets, even without fishmeal, induced good growth of carps (Meske 1976; Jayaram and Shetty 1981). Use of silkworm fecal matter-based diet on a trial basis in a rural community pond enhanced the yield of carps by 60% over the previous year (Nandeesha et al., unpublished data). As the concern in fish culture is not only growth, but also production cost, the low cost of slaughter house waste and silkworm fecal matter can be taken advantage of in developing cheaper diets for carps.

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Table 1. Proximate composition of ingredients and proportion of ingredients in three feeds.

	Composition by weight (%)											
	Dry matter		Protein		Total lipid		Ash		Pellet FM	Pellet SF	Pellet SH	
Ingredient	Batch I	Batch II	Batch I	Batch II	Batch I	Batch II	Batch I	Batch II	Batch I and II	Batch I and II	Batch I	Batch II
Fishmeal	88.44	90.80	61.32	62.10	8.20	9.10	18.40	19.20	22.50	–	–	–
Slaughter house waste	98.23	98.65	60.59	86.85	10.38	5.10	2.14	5.80	–	–	30.00	22.00
Silkworm fecal matter	84.86	85.30	39.50	37.90	3.95	3.60	1.98	2.10	–	42.50	–	–
Tapioca powder	91.75	90.42	3.68	3.20	1.20	0.80	2.67	1.30	15.00	12.50	15.00	15.00
Groundnut oil cake	93.81	94.20	43.98	46.50	11.80	12.90	5.30	5.20	22.50	19.50	20.00	20.00
Rice bran	91.54	90.80	9.31	9.80	3.00	3.90	1.88	2.10	40.00	26.00	35.00	43.00

Table 2. Proximate composition of feeds.

Parameter (%)	Pellet FM		Pellet SH		Pellet SF	
	Batch I	Batch II	Batch I	Batch II	Batch I	Batch II
Dry matter	94.80	93.80	95.90	96.40	95.20	95.80
Protein	37.30	35.80	34.90	35.40	33.10	32.66
Total lipid	12.00	11.80	9.83	8.20	7.20	7.00
Nitrogen free extract	22.10	19.45	32.07	31.06	23.50	26.14
Crude fiber	7.15	7.90	8.20	8.84	12.10	11.60
Ash	16.25	18.85	10.90	12.90	19.30	18.40
Caloric content (Kcal/g)	3.83	3.63	3.91	3.75	3.24	3.31

FM = fishmeal-based diet; SH = slaughter house waste-based diet; SF = silkworm fecal matter-based diet.

Table 3. Economics of production.

Type of feed	Cost of feed/kg (Rs.)	Total quantity of feed given (kg)	Net increase in weight (kg)	Conversion ratio	Overall survival (%)	Total cost of feed (Rs.)	Cost of production/kg of fish (Rs.)	Net fish production (kg/ha/yr)
Pellet SH	1.51	43.04	25.75	1.67	70.00	64.99	2.52	2,097.80
Pellet SF	0.97	42.02	20.67	2.03	69.13	40.76	1.97	1,684.18
Pellet FM	1.92	27.17	14.10	1.93	62.81	52.17	3.70	1,148.40

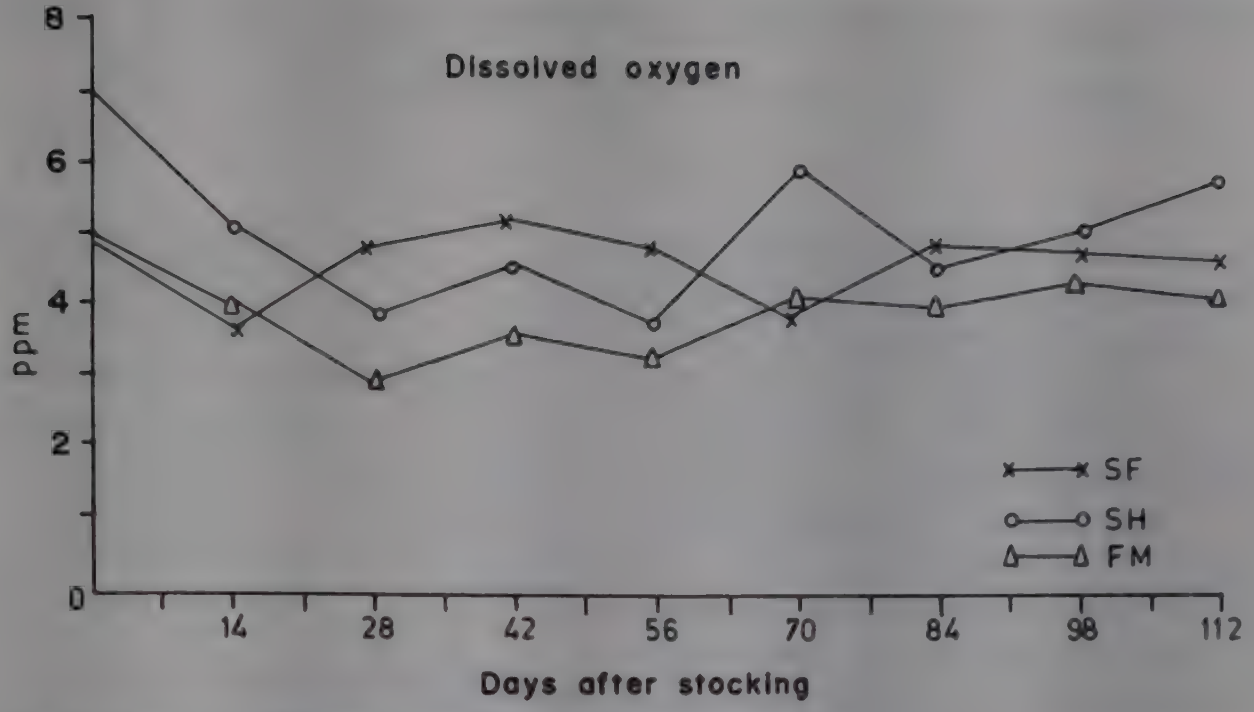

Fig. 1. Fluctuations in dissolved oxygen in different treatments.

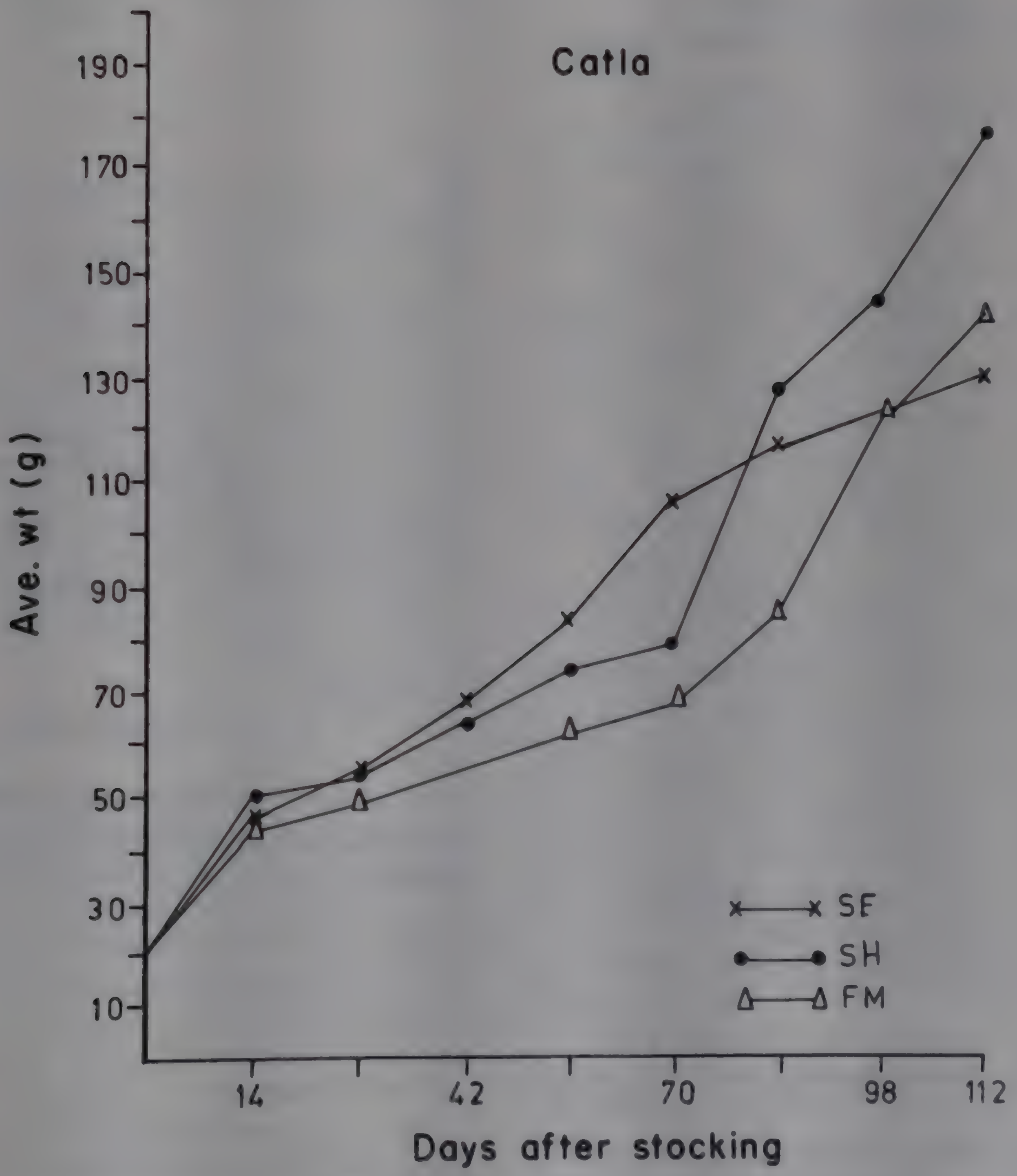

Fig. 2. Average weight attained by catla in different treatments.

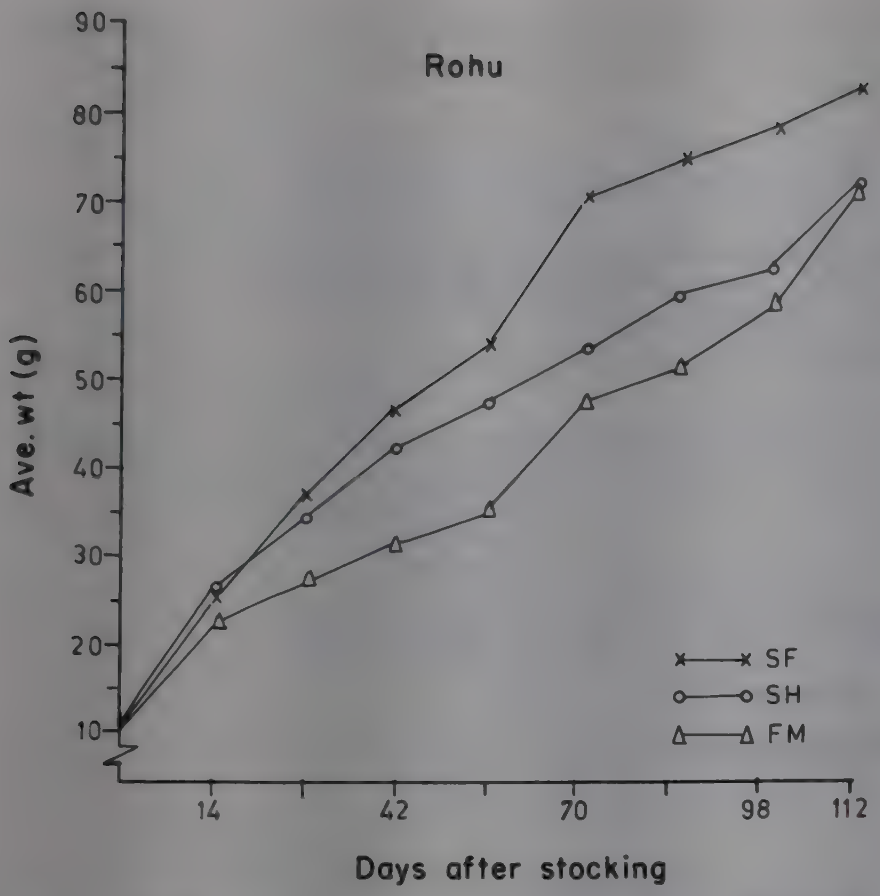

Fig. 3. Average weight attained by rohu in different treatments.

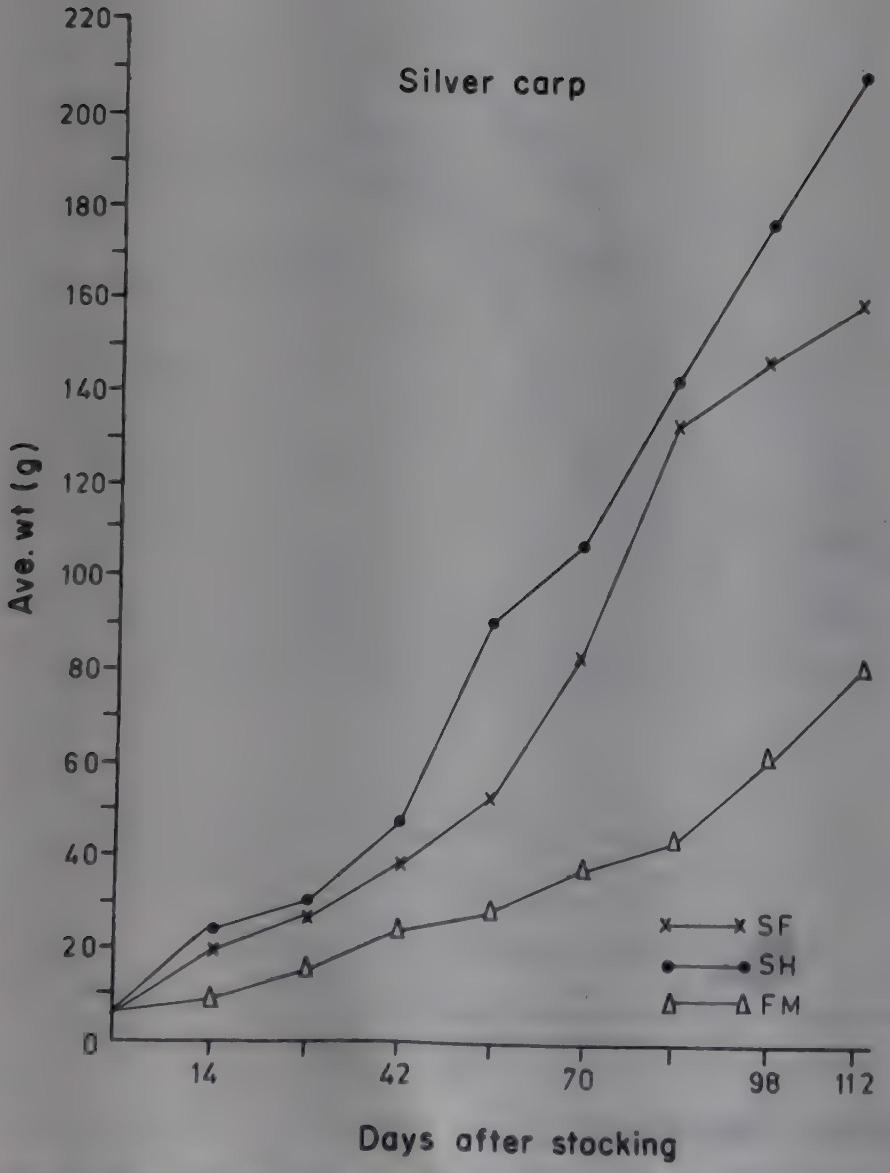

Fig. 4. Average weight attained by silver carp in different treatments.

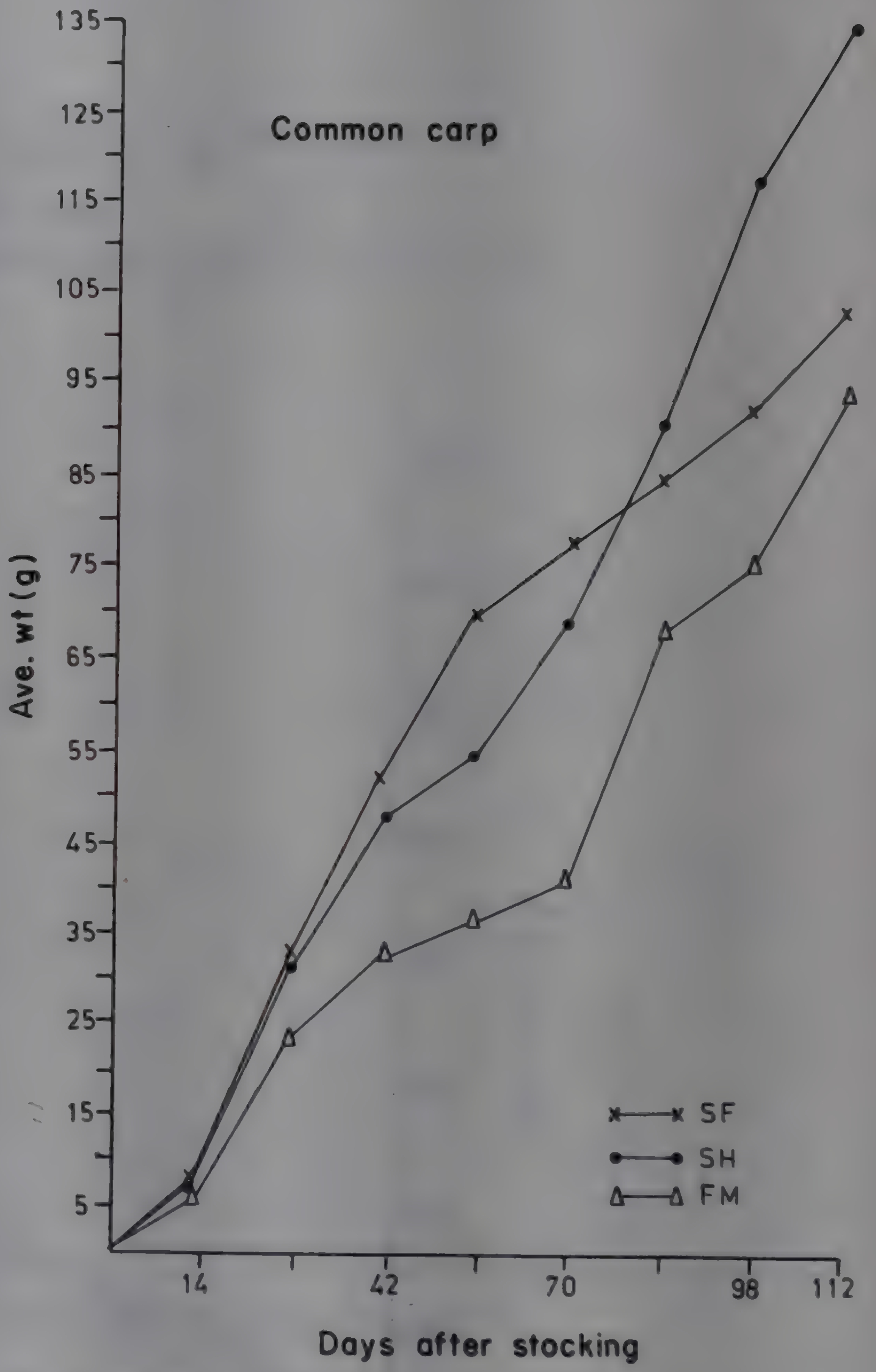

Fig. 5. Average weight attained by common carp in different treatments.

Efficiency of Some Cyanophytes as Larval Feed for Silver Carp *(Hypophthalmichthys molitrix)* and the Culture of *Spirulina platensis*

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Abstract

Six-day old carp *(Hypophthalmichthys molitrix)* fry (mean weight 3 mg) were stocked at 5/l in aquaria. These were fed with unialgal cultures of three species of blue-green algae, namely: *Anabaena* sp. *Oscillatoria quadripunctulata* and *Spirulina platensis*.

Best increase in weight was obtained when feeding consisted of *Spirulina* followed by those fed with *Oscillatoria*. Poor results were obtained when fry were fed solely with *Anabaena*. Weight gain was highest during the first two weeks with *Spirulina* as feed. On the other hand, survival of fry was higher (68%) with *Oscillatoria* than with *Spirulina* (54%). Proximate analysis of fish and algae were conducted.

Spirulina seemed to be the most promising live food organism for larval rearing of silver carp. Thus, laboratory cultures of the cyanophyte at different pH (9, 10 and 11) and two types of media (hog manure and urea) were investigated. Algal biomass production in a semi-continuous, outdoor tank system was also determined.

Introduction

There is increasing demand for fingerlings of silver carp *(Hypophthalmichthys molitrix)* in the Philippines today because of the success of the polyculture system which realized high production levels. Fingerlings of tilapia, milkfish and Chinese carp were stocked in cages in Laguna Lake, giving a total net production of as much as 25.5 t/ha (Castro et al. 1985). With this development, research efforts were directed towards optimizing conditions during larval rearing to increase fry survival and ensure availability of fingerlings for stocking.

A crucial factor in fingerling production is the supply of natural food in the environment. Live food organisms are selected on nutritive value and digestibility. Thus, for aquaculture operations, the more notable species that could be explored are the *Spirulina* spp. which contain 55-5% protein (Pirie 1975). Earlier works (Baldia et al. 1985; Pantastico et al. 1985, 1986, in press) have shown the efficiency of specific phytoplankton for fry rearing of tilapia, milkfish and bighead carp.

This paper describes the results obtained on the feeding of three cyanophytes including *S. platensis* to silver carp fry and the semi large-scale production of *Spirulina* which was demonstrated to be the most acceptable natural food for the fry .

Materials and Methods

Silver carp fry with a mean initial weight of 3 mg were stocked at 5/1 in aquaria (20-1 capacity). Fry were fed with unialgal cultures of three species of blue-green algae, namely: *Anabaena* sp., *Oscillatoria quadripunctulata* and *Spirulina platensis*.

Water was changed every other day for the first week and daily thereafter. Algal density in the rearing medium was maintained at (a) *Anabaena:* 7×10^4 - 2.6×10^6 cells/ml; (b) *Oscillatoria:* 7×10^4 -2.8×10^6 cells/ml; and (c) *Spirulina:* 1.25×10^3 - 5.0×10^3 cells/ml.

Weight measurements were recorded every two weeks. Mortality was observed daily. The pH and ammonia content of the water were also monitored.

Stock cultures of *Spirulina platensis* were maintained in Zarouk's *Spirulina* medium (Vonshak et al. 1982) consisting of 1.0 g/l each of NaCl and K_2SO_4, 3.0 g/l KNO_3, 0.08 g/l EDTA, 0.01 g/l $FeSO_4$, $7H_2O$, 0.25 ml/l H_3PO_4 and 16.8 g/l $NaHCO_3$. The medium was adjusted to pH 10.5 using NaOH. Upon reaching peak growth, usually after 14 days, *Spirulina* cultures were concentrated. These dense cultures of *Spirulina* were then reinoculated in gallon jars from the various treatments consisting of three different levels of pH (9, 10 and 11) and two types of media (organic and inorganic). Cultures were aerated vigorously and provided with illumination (3 klux) at 25-28°C.

Algal biomass was determined every three days by filtering 20 ml of sample from each treatment through a millipore filter (0.45 μm). These were oven-dried for 4 hours at 80°C and weighed. The pH was monitored every three days with an Orion pH meter.

An inexpensive medium consisting of urea, hog manure and agrimin was used for mass production of *Spirulina*. The pH was adjusted to 10.0-10.5 using NaOH flakes. Tanks (200-1 capacity) were used in the production system and placed in the covered wet laboratory at Tapao Pt. Fluorescent lights which provided continuous illumination of 3 klux were placed above the culture tanks. There was uniform circulation of algal cells due to vigorous aeration.

Harvest of algae was done every seven days. The pH, total inorganic N and PO_4-P were also monitored weekly. Nutrients were replenished and pH adjusted after every harvest. The production setup is similar to that developed for *Oscillatoria* (Pantastico et al. 1986).

Results and Discussion

Of the three species of blue-green algae used for feeding silver carp fry, *Spirulina platensis* proved to be the most acceptable (Fig. 1). Highest increase in weight of fry was obtained as early as the third week. Fry given *Spirulina* weighed 139.3 mg on the 4th week compared to those fed with *Oscillatoria* (27.1 mg) and *Anabaena* (18.2 mg). This acceleration of growth with *Spirulina* persisted throughout the eight-week experiment period. Weight increase was most pronounced during the last two weeks. Weight attained by silver carp fry fed with *Spirulina* after eight weeks was 358.5 mg which is three and six times that of the weight of fry given *Oscillatoria* and *Anabaena*, respectively.

The Duncan's Multiple Range Test on the mean weight of silver carp fry showed that those fed with *Spirulina* had significantly higher weights than those given either *Anabaena* or *Oscillatoria* (Table 1).

Weekly survival of silver carp fry was also monitored. Throughout the larval rearing period, highest survival was obtained with *Oscillatoria* followed by those given *Spirulina*. Final survival values after eight weeks showed 65.7% survival with *Oscillatoria* while 44.6% and 32% were obtained with *Spirulina* and *Anabaena*, respectively.

The mean survival rates showed no significant differences during the first week (Table 2). In general, survival values for those treatments given *Anabaena* and *Spirulina* were comparable up to the seventh week. Final survival on the eighth week showed that feeding with *Oscillatoria* gave significantly higher survival of fry.

The harvested fish from each treatment were analyzed for crude protein. Results showed that silver carp fingerlings given continuous feeding of the three species of blue-green algae did not vary significantly in crude protein content: with *Oscillatoria* 56.4%, *Anabaena* 56.16% and *Spirulina* 58.64%.

Crude protein was also highest in *Spirulina*, 68.9% CP; *Oscillatoria*, 25.60% CP and *Anabaena* 28.45% CP. The protein content of *Spirulina* grown in inorganic medium under laboratory conditions is higher than that reported in other countries (Pirie 1975).

Some of the physicochemical parameters were monitored (Table 3). Values were comparable among the different treatments except for nitrite-N which seemed to have a lower value for the *Oscillatoria* treatment.

Spirulina seems to be the best live food organism for silver carp fry. Thus, the possibility of mass producing and supplying this specific natural food for hatchery/nursery operations was investigated. The different cultural requirements of the organism in the laboratory as well as outdoors were studied.

Laboratory cultures of *Spirulina* showed that the best medium for growth is the inorganic medium with a starting pH of 10 (Fig. 2). Marked increase in algal biomass started on the ninth day up to the 12th when the yield reached 305 mg/l (dry weight) (Table 4). This high yield was sustained for about 12 more days followed by a sharp decline on the 21st day. Daily monitoring of the pH revealed that there was a gradual lowering of values from pH 10.06 to pH 8.03. When it dropped to pH 7, yield was very much reduced.

Using the same inorganic medium starting with pH 9, there was a slowing down of growth of *Spirulina* (Fig.2). The maximum yield obtained was only 70.8 mg (dry weight). This minimal growth continued for almost a month with pH fluctuations going down to 7.83.

In general, the organic medium consisting of hog manure proved inferior in growing *Spirulina* under laboratory conditions. Similar to the results obtained using the inorganic medium, pH 10 gave better growth of *Spirulina* than pH 9. Regardless of medium, pH 11 inhibited growth of *Spirulina* (Table 4). Considering the daily pH changes during the culture period, the organic medium showed wider variations than the inorganic in most cases.

Based on the results of the laboratory experiments and to lower production cost, the medium used for tank cultures of *Spirulina* was a combination of organic and inorganic fertilizers with a starting pH of 10. A semicontinuous algal production system earlier established for *Oscillatoria* (Pantastico et al. 1986) was also used.

Semi large-scale production of *Spirulina* in tanks (120-l capacity) reached its peak after 35 days with a total harvest of 187.9 g (dry weight) or 1.56 g/l (Table 4, Fig. 3a). On a daily basis, this is computed as 44.5 mg/l/day. The growing period was extended to 49 days. The nutrients were then replenished. Water analysis at this time also showed that the total inorganic nitrogen and PO_4-P had very low values at 210 ppb and 760 ppb, respectively (Fig. 3b). Wide fluctuations in the nitrogen and phosphorus content of the water occurred during the entire experimental period. With the replenishment of the medium, there was a revival of growth, so that the culture of *Spirulina* was maintained up to 70 days.

Conclusions

It may be concluded that six-day old silver carp fry showed preference for the cyanophyte, *Spirulina platensis*. Earlier reports (Jhingran and Pullin 1985) indicated that two- to three-day old hatchlings of silver carp feed on zooplankton primarily while older fry up to the adult stage accept both zooplankton and phytoplankton with preference for the latter. Our study confirmed the acceptability of phytoplankton to very young silver carp fry. Similar results were obtained with tilapia fry given the unicellular species of *Navicula notha* and *Chroococcus dispersus* as live food (Pantastico et al. 1985). On the other hand, assimilation rate studies using ^{14}C-labelled live food organisms showed that acceptability of phytoplankton to bighead carp larvae occurred at a later stage (Baldia et al. 1985).

It is noteworthy that even among members of Cyanophyta, certain species proved better feeds than others. Increase in weight of silver carp fry was significantly high ($p < 0.05$) in the treatment with *Spirulina* compared to those fed with *Oscillatoria* and *Anabaena*. The beneficial effect of *Spirulina* feeding may be explained in terms of its high nutritive value and absence of toxic effects (Ciferri 1983).

Spirulina production for growing silver carp fry under Philippine conditions seems a bright possibility. The culture is stable for as long as 14 days. Moreover, utilization of animal manures in the production scheme will reduce cost tremendously. It is envisioned that a village-level technology for algal biomass production will be developed to support aquaculture operations in the country.

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Table 1. Duncan's multiple range test on the mean weight (g) of silver carp fry fed with three cyanophytes.

	Weeks			
Treatments	2	4	6	8
Anabaena	0.0131^{b}	0.0182^{b}	0.0265^{b}	0.0590^{b}
Oscillatoria	0.0172^{ab}	0.0270^{b}	0.0560^{b}	0.1195^{b}
Spirulina	0.0224^{a}	0.1393^{a}	0.203^{a}	0.3585^{a}

Means with the same superscript in each row are not significantly different ($p < 0.05$).

Table 2. Duncan's multiple range test on the mean survival rate (%) of silver carp fry fed with three cyanophytes for eight weeks.

	Weeks							
Treatments	1	2	3	4	5	6	7	8
Anabaena	95.0^{a}	71.3^{ab}	60.0^{b}	54.6^{b}	50.3^{b}	47.3^{b}	44.67^{b}	32.0^{c}
Oscillatoria	93.3^{a}	80.3^{a}	76.0^{a}	74.0^{a}	70.0^{a}	68.6^{a}	66.33^{a}	65.7^{a}
Spirulina	93.0^{a}	60.6^{b}	57.6^{b}	56.6^{b}	55.3^{b}	54.3^{b}	50.3^{b}	44.7^{b}

Means with the same superscript in each row are not significantly different ($p < 0.05$).

Table 3. Range of physicochemical parameters.

	Parameters			
Treatments	Dissolved oxygen (ppm)	pH	Ammonia-N (ppb)	Nitrite-N (ppb)
Anabaena	2.0 – 7.9	8.0 – 8.2	28.8 – 86.3	8.8 – 397
Oscillatoria	2.3 – 7.7	8.0 – 8.3	29.3 – 348	6.0 – 407
Spirulina	2.3 – 7.4	7.8 – 8.3	23.6 – 256	8.9 – 184

Table 4. Growth of *Spirulina* in organic (OM) and inorganic (IM) media with initial pH of 9, 10 and 11.[1]

	Treatments											
	IM-9		OM-9		IM-10		OM-10		IM-11		OM-11	
Growth period (days)	pH	Wt. of *Spirulina* (mg. dry wt./l)	pH	Wt. of *Spirulina* (mg. dry wt./l)	pH	Wt. of *Spirulina* (mg. dry wt./l)	pH	Wt. of *Spirulina* (mg. dry wt./l)	pH	Wt. of *Spirulina* (mg. dry wt./l)	pH	Wt. of *Spirulina* (mg. dry wt./l)
0	9.05	0.086	9.08	0.086	10.02	0.086	10.05	0.086	11.01	0.086	11.06	0.086
3	9.39	16.73	8.49	6.90	10.66	23.97	9.72	1.03	10.98	—[2]	11.02	—[2]
6	10.13	21.80	7.88	7.65	10.42	33.80	9.30	1.47	10.96	—	11.01	—
9	10.19	35.2	7.42	10.97	10.06	114.10	7.16	4.30	10.98	—	11.02	—
12	9.19	38.96	8.12	4.00	9.24	305.30	8.25	3.73	—	—	—	—
15	8.18	70.80	8.09	3.10	8.25	292.20	8.29	13.40	—	—	—	—
18	8.16	64.3	7.98	12.30	8.03	198.30	8.19	32.50	—	—	—	—
21	8.10	65.2	7.63	8.40	7.85	72.40	8.16	28.60	—	—	—	—
24	7.95	63.6	7.57	9.40	7.66	75.30	8.17	24.40	—	—	—	—
27	7.83	64.7	7.86	8.84	7.75	82.40	8.17	26.30	—	—	—	—

[1] Data are means of three replicates.

[2] No growth.

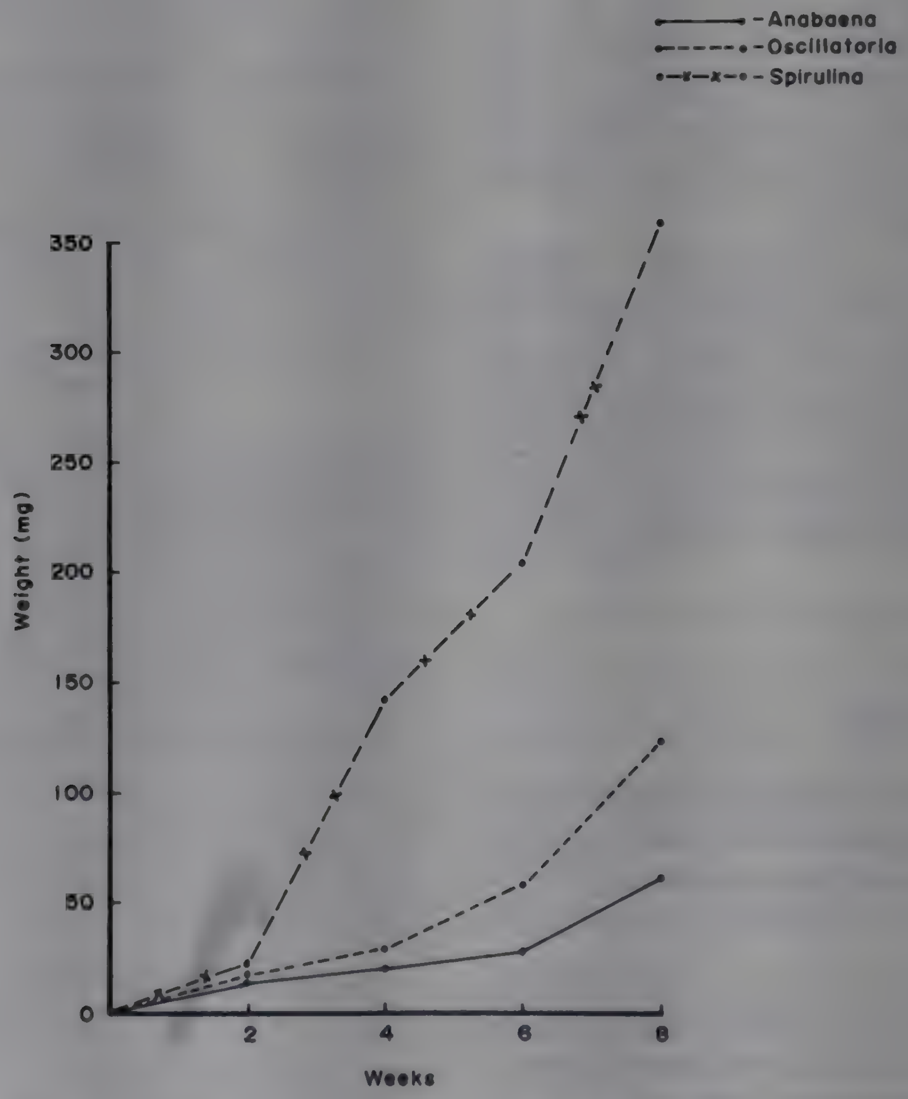

Fig. 1. Growth of silver carp fry fed with three species of blue-green algae.

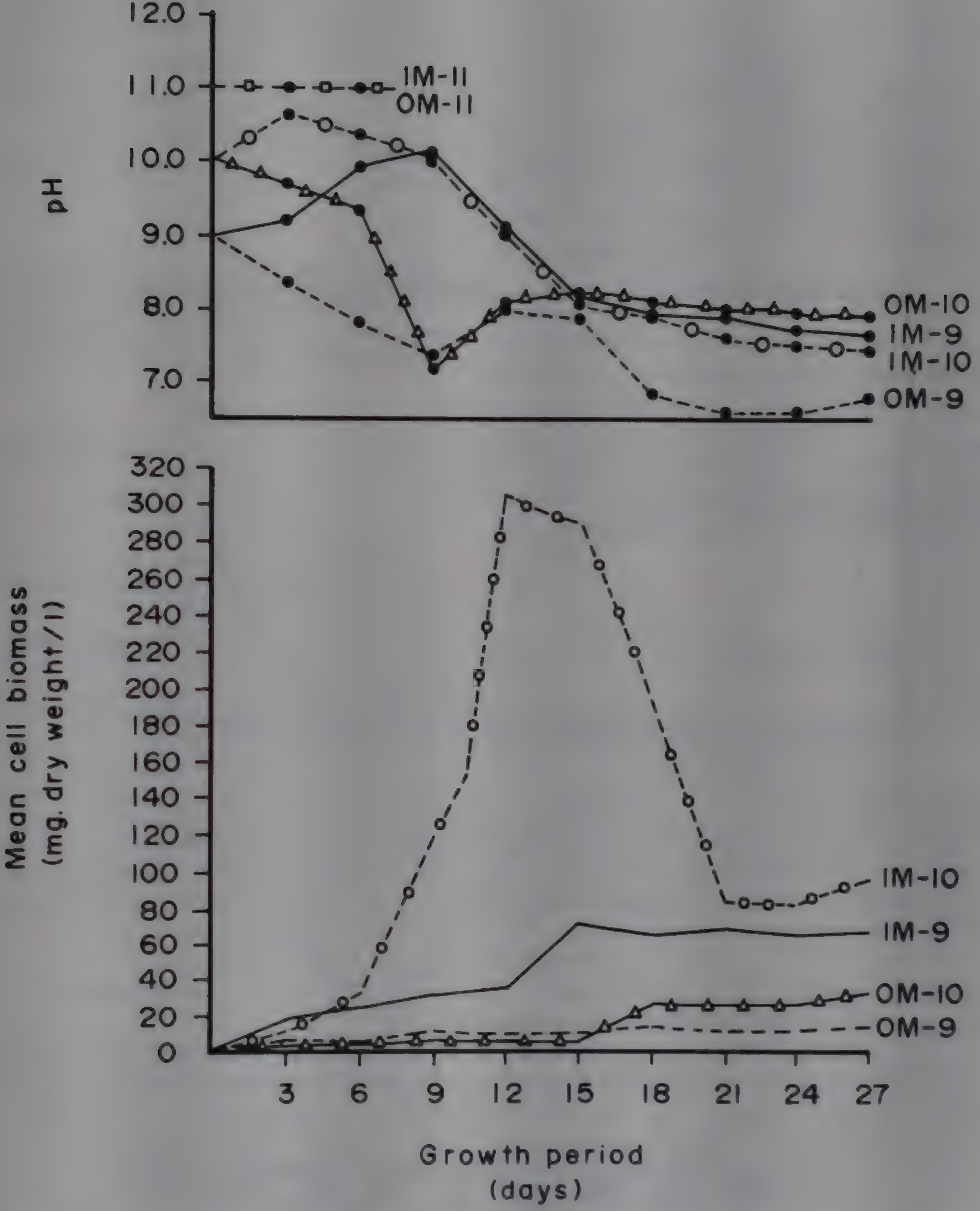

Fig. 2. Growth of *Spirulina* in Inorganic (IM) and Organic (OM) media with initial and corresponding pH.

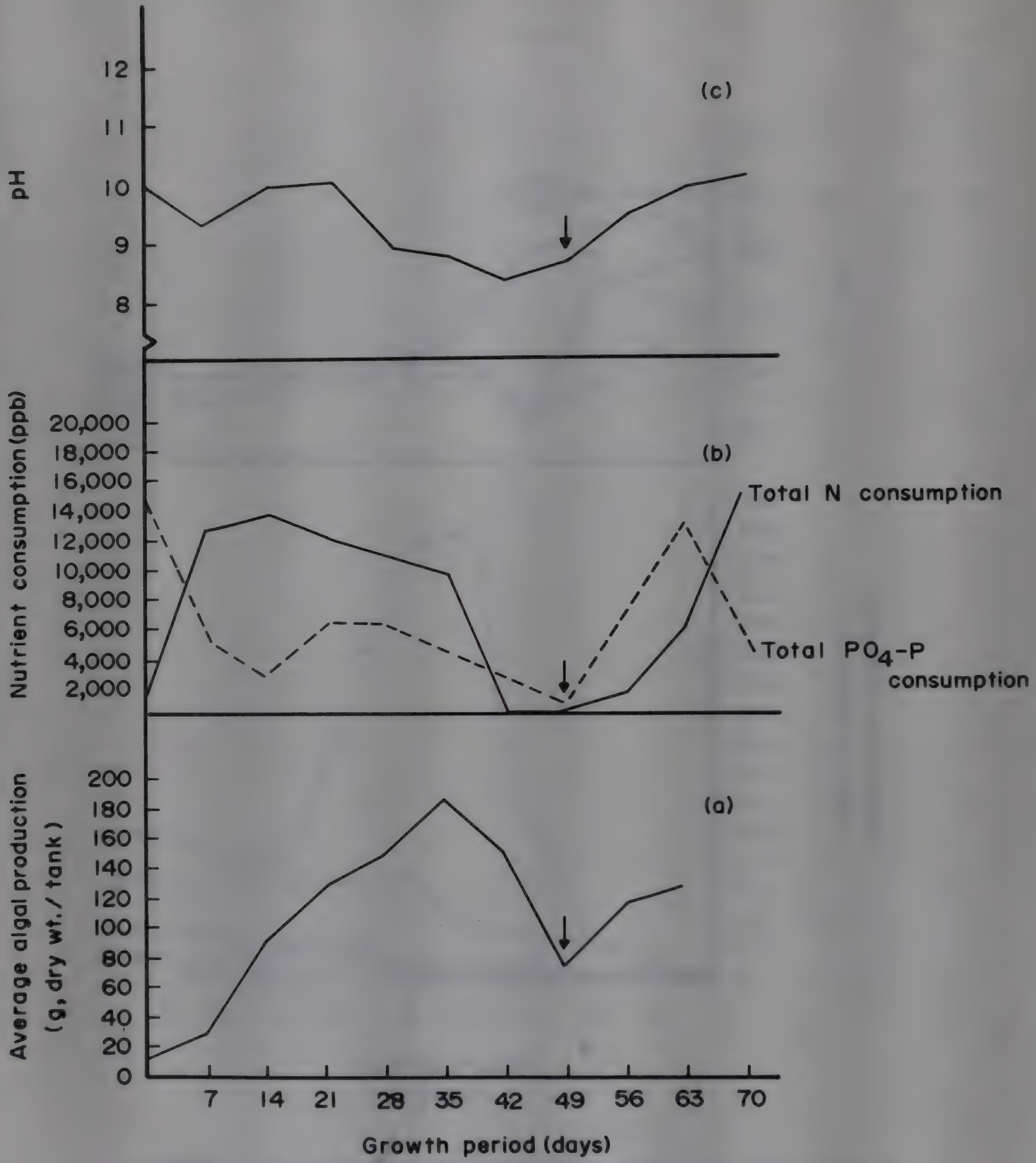

Fig. 3. Semilarge-scale production of *Spirulina* (a), nitrogen and phosphorus consumption (b) and pH (c) in medium with the combination of organic and inorganic fertilizers. Arrows indicate harvest and replenishment.

Effect of Supplemental Lecithin and Lipid Sources on the Growth and Survival of *Penaeus monodon* Juveniles*

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Abstract

Penaeus monodon juveniles were reared in 60-liter fiberglass oval tanks in a flow-through seawater system for eight weeks to determine the effect of lecithin and type of lipid on growth and survival. Nine isonitrogenous and isocaloric diets consisting of a basal practical diet (40% protein, 10% lipid) with three levels of soy lecithin and three sources of lipid cod liver oil, crude degummed soybean oil and purified soybean oil were used. Feed was offered twice daily. Percentage weight gains significantly increased ($P < 0.05$) as the level of lecithin was increased from 0 to 2% regardless of the lipid source. At all levels of lecithin, survival rates were significantly higher in those fed diets containing crude degummed soybean oil compared to those fed either cod liver oil or purified soybean oil. Lecithin levels and lipid sources did not significantly affect the feed conversion values. The best diet was that which had 2% soy lecithin with 3.8% crude degummed soybean oil.

Introduction

Lecithin, composed of phosphatides and other polar lipids and carbohydrates, has been found to be required by some crustaceans such as *P. japonicus* (Deshimaru 1981) and spiny lobster, *Homarus americanus* (Conklin et al. 1980; 'D'Abramo et al. 1981). Catacutan and Kanazawa (unpublished data) observed that 1% soybean lecithin in the diet for P. monodon juveniles gave better survival than fish or chicken egg lecithin in semipurified diets. *Macrobrachium rosenbergii* did not require lecithin in semipurified diets containing casein and gelatin as sources of protein. However, best weight gain was obtained on a practical type diet (Hilton et al. 1984).

Conklin et al. (1980) reported that spiny lobsters need around 7.5% soy lecithin in purified diets with casein as the protein source. On the contrary, when crab protein was used as a protein source, Castell et al. (pers. comm.) found no need for lecithin in the diets of *H. americanus*.

Cod liver oil, pollack liver oil, corn, soybean, coconut, sunflower seed, linseed and wheat germ oils have been tried in diets for various crustaceans (Colvin 1976; Deshimaru et al. 1979; Deshimaru 1981; Mendoza 1982; Teshima and Kanazawa 1985; Catacutan and Kanazawa 1985; Bautista 1986).

Crude degummed soybean oil has not been tried in *P. monodon* juvenile diets while cod liver oil and soybean oil, singly or in combination, have been tried in practical diets for *P. monodon* (Mendoza 1982; Bautista 1986). However, their effects with various levels of soybean lecithin have not been elucidated.

This study aimed to determine the effect of increasing levels of supplemental lecithin and the type of lipid incorporated in pelleted practical diets of *P. monodon* juveniles.

Materials and Methods

The experiment was undertaken on a 3 x 3 factorial designed for random tests and ran with five replicates. A two-way analysis of variance was used.

Six juvenile *P. monodon* with mean weight of approximately 0.090 g were stocked at random and reared in 40 l of seawater contained in 60-l oval fiberglass tanks. Aeration through airstones and seawater were provided in a flow-through system for eight weeks under laboratory conditions.

The animals were weighed individually prior to stocking and henceforth weighed every 14 days. Feed was offered twice a day at 25% of body weight and was later decreased to 20% in the last month of the experiment.

Salinity and temperature were monitored every morning and afternoon. The tanks were cleaned daily by siphoning out fecal matter and left-over feed. Thorough cleaning of the tanks was done once a week.

All feedstuffs were kept constant except for the source of lipid and the levels of lecithin (Table 1). Diets 1 to 3 contained cod liver oil, and 0, 1 and 2% Stasol-U.F., a brand name of a form of soybean lecithin. Diets 4 to 6 had crude degummed soybean oil, and 0, 1 and 2% soybean lecithin while Diets 7 to 9 contained purified soybean oil with 0, 1 and 2% soy lecithin, respectively.

The diets were isonitrogenous and isocaloric. Lecithin was incorporated with the oils before addition to the premixed dry feedstuffs. Water was added to form a dough which was extruded through a garlic press. The extrusions were steamed for 5 minutes, dried at 60°C and kept at around 4°C in a refrigerator in plastic covered bottles. Proximate analyses of the diets (Table 2) were carried out as follows: crude protein was analyzed in a Kjeltec autoanalyzer 1030, crude lipid was extracted in a Soxtec, crude fiber was done in a Fibertec system, crude ash was done by AOAC (1975) while moisture content was determined in a Mettler Moisture Determination Balance LP15. Nitrogen-free extracts were computed by arithmetical difference.

Prior to fatty acid analysis, total lipids were extracted by Bligh and Dyer (1959) method as modified by Kates (1972). Fatty acid composition of each diet (Table 3) was determined by gas-liquid chromatography on a Shimadsu GC-4C gas chromotograph with an FID detector. Literature for published oils (Ackman and Burger 1965) and valid standards were used for the identification of FAME. Neutral and polar lipid contents of the oil were determined by column chromatography on silicic acid.

Results and Discussion

Weight gain was significantly affected by levels of lecithin rather than by source or type of oil (Table 3). In all instances, regardless of type of oil, the addition of 1% soy lecithin in the diets increased weight. Likewise, further increase in weight was observed when 2% soy lecithin was added to the diets. Total lecithin analyses of the diets showed that in diets where no lecithin was added, diet nos. 1, 4 and 7, lecithin from the feedstuffs such as shrimp meal and rice bran, amounted to 0, 87; 1, 07 and 0, 87, respectively. Thus, all the diets contained lecithin and total analyzed levels were approximately 1% higher than amounts of lecithin added to the diets (Table 2).

Feed conversion was generally poor (Table 3). Trends indicate feed conversion in diets with crude degummed oil were relatively better followed by those with cod liver oil and was poorest in those given refined soy bean oil (Table 3). As lecithin levels increased in the diet, feed conversion improved and was pronounced in diets with crude degummed oil.

Little is known of the lecithin needs of *P. monodon*. When vitamin-free casein, wheat gluten, and spray-dried egg white were used as protein sources in the diet of lobsters, *H. americanus*, Conklin et al. (1980) and D'Abramo et al. (1981) concluded that soy lecithin and phospholipids in the diet provided for high survival.

Their results suggest that phosphatidyl choline and/or phosphatidyl inositol are highly effective in enhancing growth and survival of *H. americanus* juveniles. Teshima et al. (1982) showed that 3% soybean phosphatidyl choline and 1% cholesterol are needed by *P. japonicus* larvae in a semi-purified diet containing 6% pollack liver oil.

On the contrary, Hilton et al. (1984) reported that *Macrobrachium rosenbergii* did not need supplemental lecithin when given semi-purified diets for 12 weeks. In the present study, survival did not improve with supplemental lecithin in the diet. However, weight gain was definitely improved with added lecithin.

According to Liu et al. (1974) in Hilton et al. (1984), crustaceans, in general, can synthesize phospholipids such as phosphatidyl choline via pathways similar to those of mammalians. Lee and Puppione (1978) reported that the phospholipid is the principal transport form of lipids in crustacean hemolymph. Teshima and Kanazawa (1979) suggested that phospholipids are required for lipid transport in *P. japonicus* and are very important in diets for this species.

Numerically, lower values for survival were observed when lipid sources were decreased from 7 to 3% (Table 4). Lecithin that was used contained around 63% phosphatides. Phosphatides may be necessary for lipid transport in *P. japonicus* (Teshima and Kanazawa 1979). Oil was important not only for the essential fatty acids but also as an energy source. Bautista (1986) showed that *P. monodon* juveniles need from 5 to 10% lipid in their diets while Mendoza (1982) reported that a dietary lipid level of around 12% was required by *P. monodon* juveniles for maximum growth, efficient feed conversion and optimum survival rate. Clarke and Wickins (1980) showed that in *Penaeus merguiensis*, the major lipid classes are phospholipids (47%), free sterol (27%) and triacyglycerol (197%). Animal lipid is probably an essential component in the diet if the species is to maintain a normal lipid and fatty acid composition. They also concluded that diets high in $20{:}5\omega 3$ and $22{:}6\omega 3$ are probably necessary to maintain a wild-type fatty acid composition in cultured penaeids like *P. merguiensis*. Likewise, Kanazawa et al. (1979) reported that *P. monodon* and *P. merguiensis* probably require $18{:}3\omega 3$, $20{:}5\omega 3$ and $22{:}6\omega 3$ for normal growth.

Survival of *P. monodon* juveniles were significantly affected by the type of oil rather than the level of lecithin in the diet (Table 3). Animals fed crude degummed soybean oil had the highest survival rate compared to those fed cod liver oil or purified soybean oil regardless of the level of supplemental lecithin. Those fed cod liver oil and purified soybean oil had similar survival rates.

Due to cod liver oil, diets 1 to 3 were high in $\omega 3$ fatty acids whereas those that contained crude degummed soybean or purified soybean oil contained more of the $\omega 6$ fatty acids (Table 4). Hence, the $\omega 3$ to $\omega 6$ ratios for the diets containing cod liver oil were generally higher for diets 1 to 3 and lower for diets 4 to 9. The higher survival rate obtained with crude degummed oil can not be explained in terms of fatty acid composition of the diet. Crude degummed soy oil contains more of the ω6 than the ω3 series and vice versa for cod liver oil, yet survival rate was higher in those fed diets with crude degummed oil. There is the possibility that fish that thrive in tropical waters may need 6 as well as 3. *Tilapia zillii* was found to need more ω6 than ω3 (Kanazawa et al. 1980). Catacutan and Kanazawa (1985) reported that ω6 in fatty acids were present in both neutral and polar lipid of *P. monodon* juveniles fed a lipid free diet in the amounts of 10.83% and 16.13%, respectively. They also reported high levels of polyenoic acids 20:4ω6, 20:5ω3 and 22:6ω3. The ω3 series were high in the polar lipid fraction and preferably stored in the polar fraction. The report of Teshima et al. (1976) show differing levels of ω6 and ω3 in various crustaceans. Furthermore, crude degummed soybean oil could have contained other nutrients that were removed in the processing of purified soybean oil.

Crude degummed oil contained 11.74% polar lipid while cod liver oil and refined soybean oil contained 14.7% and 6.09%, respectively. Thus, assuming that most of the ω3 fatty acids are found in the polar lipid fraction (Catacutan and Kanazawa 1985), it is not surprising that crude degummed soya oil gave better survival than purified soybean oil. Neutral lipid was 88.3% for crude degummed soybean oil, 85.3% for cod liver oil and 93.9% for purified soya oil. According to Deshimaru (1981), polar lipids are more important in the diet of *P. japonicus* than neutral lipids.

Up to the present, the active component of lecithin remains to be unidentified. Although crude degummed soybean oil may not contain enough of the polyenoic ω3 series of fatty acids present in prawn tissue, it is a source of energy and could lower the cost of the feed. Deshimaru et al. (1979) showed that a mixture of pollack liver oil and soybean oil in a 3:1 to 1:1 ratio containing approximately 20-30% ω6 and 10-20% ω3 fatty acids is desirable for *P. japonicus* and a suitable lipid level of 6% has been found feasible as feedstuff in practical diets for prawns.

Based on the results of this study, crude degummed soybean oil is a better source of oil than purified soybean oil and 2% supplemental lecithin is recommended in diets for *P. monodon* juveniles.

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*SEAFDEC Aquaculture Department Contribution No. 183.

Table 1. Percentage composition of the diets.

Ingredients/diet no.	1	2	3	4	5	6	7	8	9
Peruvian fish meal	30.00	30.00	30	30	30	30	30	30	30
Shrimp meal (*Acetes* sp.)	15.00	15.00	15	15	15	15	15	15	15
Soybean meal (defatted)	15.00	15.00	15	15	15	15	15	15	15
Rice bran	15.00	15.00	15	15	15	15	15	15	15
Wheat flour (All purpose)	10	10	10	10	10	10	10	10	10
L-Potato starch	5.00	5.00	5	5	5	5	5	5	5
Cod liver oil	7.00	5.41	3.82	—	—	—	—	—	—
Soy lecithin (Staasl UF)	0.00	1.59	3.18	0.0	1.59	3.18	0.0	1.59	3.18
Crude degummed soybean oil				7.0	5.41	3.82			
Purified soybean Oil (soyalite)							7.0	5.41	3.82
Vitamin mix[1]	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50
Mineral mix[2]	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50

[1] Composition of vitamin mix (g/100 g vitamin mix):
Thiamin HC1 0.15, Riboflavin 0.50, Pyridoxine 0.15, Nicotinic acid 2.00 Capanthothenate 0.75, Inositol 10.00, Biotin, 0.015, Folic acid 0.0375, Para amino benzoic acid 1.00, Choline chloride 20.00, Ascorbic acid 25.00, L-tocopherol 1.00, Menadione 0.10, B-carotene 0.67, Ergocalciferol 0.015, Cyanocobalamine 0.001, Cellufil 38.61.

[2] Composition of mineral mix:

K_2HPO_4	10.00
$NaH_2PO_4.H_2)$	18.92
$Ca(H_2PO_4).H_2O$	26.50
Calactate	16.50
Kcl	2.80
$MgSO_4.7H_2O$	10.00
Fe citrate	1.20
Trace metals*	
Cellufil	13.08

*Trace metals	%
$Alc1_3.6H_2O$	0.024
$ZnSO_4.7H_2O$	0.476
$MnSO_4.H_2O$	0.081
$Cuc1_2$	0.020
KI	0.023
$Cocl_2.6H_2O$	0.140
Cellufil	

Table 2. Proximate analyses+ and lecithin* content of the diets in percentages on a dry matter basis.

Diet no.	1	2	3	4	5	6	7	8	9
Moisture	7.98	8.64	8.47	8.42	8.97	8.44	8.58	8.51	8.44
Crude protein	39.73	40.04	40.13	39.69	40.20	40.06	40.26	40.07	39.64
Crude fat	10.54	9.42	10.13	10.42	10.40	10.76	10.60	9.50	9.62
Crude fiber	4.18	4.74	3.92	4.20	4.20	4.31	4.30	4.36	4.35
NFE	30.60	30.85	31.00	30.69	30.40	30.20	29.74	31.11	31.55
Ash	14.88	14.85	14.92	15.00	14.80	14.86	15.10	14.98	14.94
Lecithin	0.87	1.82	2.82	1.07	1.89	2.90	0.87	1.84	2.90

+Analyzed by Centralized Analytical Lab. SEAFDEC, AQD.
*Analyzed by Woodson-Tenet Lab. Inc., Memphis, Tennessee, U.S.A.

Table 3. Percentage weight gain, survival and feed conversion of *P. monodon* juveniles reared on various levels of lecithin and lipid sources.

Diet no.	Lipid source	Lecithin %	Weight gain %	Survival %	Feed conversion
1	Cod liver oil	0	385.56^a	60^a	12.73
2		1	386.46^a	57^a	11.03
3		2	484.56^b	47^a	11.80
4	Crude degummed soya oil	0	292.12^a	77^b	11.27
5		1	370.53^a	67^b	9.80
6		2	622.83^b	80^b	7.45
7	Purified soybean oil	0	264.88^a	47^a	12.32
8		1	381.39^a	47^a	12.18
9		2	578.33^b	43^a	13.72
			S.E. ± 49.02	S.E. ± 6.86	S.E. ± 5.27

*Number with the same superscripts are not significantly different from each other ($P < 0.05$).

Table 4. Fatty acid profile of the diets in percentages.

Diet no.	1	2	3	4	5	6	7	8	9
C14:0	4.30	1.51	2.63	0.45	0.63	0.71	0.97	0.84	0.88
15:0			0.28		0.08				0.14
16:0	11.49	14.08	22.08	12.36	11.83	11.53	15.73	15.04	14.19
16:0	17.31	9.27		0.19					
17:0	0.59		1.42						0.79
18:0			0.86		0.04				
18:1	23.44	29.35	28.15	29.28	29.14	27.23	28.09	24.04	25.61
18:2ω6	15.88	16.25	23.80	44.24	46.08	45.53	43.79	47.59	45.76
18:3ω6		1.0	2.72	5.81.	6.1	5.56	6.68	9.04	7.09
18:3ω3	6.74	9.59	6.67	1.34	1.34				1.87
20:1		0.44	0.42	0.26	0.52			0.14	0.34
20:4ω6			0.15	1.80					
22:1		3.89							
20:5ω3	11.87	9.19	5.02	1.24	2.01	1.40	4.33	1.15	1.76
22:4ω6	3.93	1.51	4.78					0.37	0.13
22:5ω6		0.08	0.39	1.13	0.18				
22:5ω3	1.52	1.76	0.28		1.04	1.76		0.89	1.27
22:6ω3	2.61	2.04	2.45	1.58	0.81	2.16	1.81	0.48	0.17
Total ω3	23.04	22.58	11.94	4.16	5.2	5.34	6.14	2.32	5.07
Total ω6	19.81	18.84	14.42	52.98	52.36	51.09	50.47	57.06	52.98
ω3/ω6 ratio	1.61	1.20	2.21	0.078	.099	.104	.122	0.04	0.096

Fat-Soluble Vitamin Requirements of the Rotifer *Brachionus plicatilis*

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Abstract

The use of yeast in combination with marine *Chlorella* as food for mass production of the rotifer *Brachionus plicatilis* has grown popular in recent years. It has been discovered, however, that washed cells of baker's yeast have no nutritive effect on the population growth of the rotifer when cultured axenically. In this study, the effects of three fat-soluble vitamins (Vitamins A, D and E) on its population growth were evaluated by axenically culturing the rotifer in several types of food suspensions. One type was the basic food suspension (control), consisting of yeast suspension fortified with Vit. B_{12} and the others were the basic food suspension plus the vitamins (variables). The effects of the three vitamins supplemented at various concentrations alone and combined were determined with and without Vit. B_{12}. The three fat-soluble vitamins all showed supplementary effect. However, toxicity was exhibited when given at high concentrations. Combining the three vitamins was observed to significantly increase the population growth of the rotifer. The fact that Vit. B_{12}-free food suspensions showed almost no growth confirmed that Vit. B_{12} is an essential nutrient for the growth of the rotifer.

Introduction

The success in culturing commercially important fish in Japanese mariculture can be attributed mainly to the development of advanced techniques in mass production of the rotifer, *Brachionus plicatilis*. However, problems encountered in mass culture of *Chlorella*, the main food of the rotifer, demand the development of an artificial diet which could stabilize rotifer supply. This implies the need to investigate the nutritional requirements of the rotifer.

Today, baker's and marine yeasts are being used extensively in combination with *Chlorella* as food for mass production of the rotifer. However, washed cells of baker's yeast have no nutritional effect on the population growth of the rotifer when cultured axenically (Hirayama and Funamoto 1983). Success in mass production of the rotifer with yeast could be due to the nutritive elements released during the decomposition of yeast as well as to the phytoplankton and bacteria growing in the culture tanks utilizing the decomposed products as a nutritive source (Hirayama and Watanabe 1973). Previous investigations by Scott (1981) and Hirayama and Funamoto (1983) showed that Vit. B_{12} is an essential nutrient for the growth of the rotifer.

In this study, the supplementary effects of three fat-soluble vitamins (Vits. A, D and E) on the population growth of the rotifer were evaluated under bacteria-free conditions, by adding them to the basic food suspension, which consists of baker's yeast and Vit. B_{12}.

Materials and Methods

The first-laid eggs collected from an actively growing group of rotifers were divided into several groups and axenically cultured in several types of food suspensions. The basic (control) food suspension consisted of baker's yeast cells at 200 μg/ml and of Vit. B_{12} at 1.4 μg/ml. The baker's yeast used for the experiment had been cultured in a 200-ml flask containing Mayer's medium with continuous aeration after purification by picking up its colony from an agar plate culture. Cells employed were only those under the exponential stage of growth, washed by centrifugation and then resuspended in sterilized diluted seawater fortified with Vit. B_{12}. The seawater used for the experiments had been treated with activated charcoal to eliminate organic substances. The fat-soluble vitamins were added to the basic food suspensions in an emulsified state.

The rotifers used for the experiment were amictic females derived from the same strain (large type) employed in previous investigations (Hirayama and Ogawa 1972). During the experiment, temperature was maintained at 23^{o}C and no occurrence of mictic females or males was observed. The food suspensions were stirred with the Circle Shaker set at 130 rotations/min. for 15 min. six times a day to keep the yeast cells in a suspended state throughout the investigation.

The indices for population growth of the rotifer were determined by using batch culture and individual culture methods.

In batch culture method, the offspring which hatched out in one day from collected first-laid eggs were cultured in the food suspensions. In each test tube, five individuals were placed and were cultured for a period of seven days. During the cultivation, no extra food was added into the tubes. After the culture period, the increase in the number of rotifers was determined.

In individual culture method, first-laid eggs were separately cultured in several tests tubes by inoculating each with two individuals, with 12 to 14 individuals for each experimental group at the start of the culture period. They were observed daily with the renewal of food suspensions, to count the number of surviving individuals and the number of eggs laid. From daily survival and fecundity rates thus obtained, two indices - the intrinsic rate of population increase (r) and the net reproduction rate (Ro) were calculated on the basis of Birch's computational method (1948). Assuming that the experimental group grew according to the fecundity rate obtained from the individual culture method, (Ro) implies the number of eggs laid by an average female during its lifespan or the rate of multiplication in one generation. Intrinsic rate of population increase (r) is defined as the constant in the differential equation of population increase, $dN/dt = rN$, in an unlimited environment (N, number of individuals and t, time elapsed in days from putting the eggs into the test tubes).

The effects of the addition of the vitamins to the basic food suspension on the population growth of the rotifer were evaluated by comparing the ratio of the indices r and Ro obtained in the experimental suspensions to those in the basic food suspensions (relative r and relative Ro), respectively. When the relative r and Ro were greater than one, the vitamins were assumed to enhance growth.

Evaluation of the effects of each vitamin at various concentrations was first determined using the individual culture method. The combined effect of the three vitamins was evaluated in both batch and individual culture methods by investigating different combinations at the concentrations which showed the highest promoting effect on growth in the tests for the individual vitamins. The combined effect of the three vitamins at half optimum concentrations was also evaluated in the two culture methods. The supplementary effect of each of the three vitamins when Vit. B_{12} was removed was investigated in the batch culture method.

Fat-soluble vitamins evaluated for supplementary effects were products of the Wako Pure Chemical Industries Ltd. Procedures for the collection of first-laid eggs, sterilization using antibiotic mixtures, the preparation of food suspensions and the sterility test using STP medium are the same as those employed by Hirayama and Funamoto (1983).

Results

Table 1 shows the individual effect of each vitamin at various concentrations using the individual culture method. Their relative r and Ro are shown in Fig. 1. For Vit. A, the greatest relative indices for population growth were observed at 2.0 μg/ml. At concentrations greater than 2.0μg/ml, lower indices were observed. The same tendency was observed for Vits. D and E. The greatest relative indices were observed at 0.2μg/ml and 1.0 μg/ml of Vit. D and Vit. E, respectively.

The combined effects of the vitamins using the two culture methods are shown in Fig. 2 and Table 2. The food suspension containing the three types of vitamins in a combined state showed higher increase in individual number compared to that of the basic food suspension and those supplemented with only one or two types of vitamins (Fig. 2). Combining the three types of vitamins also showed the highest indices (Table 2).

Rotifers cultured in Vit. B_{12}-free food suspensions showed lower growths compared to that in the basic food suspension (Fig. 3).

Discussion

The three fat-soluble vitamins, A, D and E, all showed supplementary effects on the population growth of the rotifer. However, these vitamins exhibited toxicity when given at higher concentrations. The combined vitamins greatly enhanced population growth of the rotifer compared to individual vitamin supplements. Even reducing the concentrations to half of their strength showed higher growth of the rotifers than those supplemented with only one type of vitamin. Poor or almost no growth was observed from the rotifers cultured in Vit. B_{12}-free suspensions provided with the fat-soluble vitamins. This result supports previous reports that Vit. B_{12} is an essential nutrient for the population growth of the rotifer (Scott 1981; Hirayama and Funamoto 1983).

The values of the indices obtained for all the above variables are still much lower than those obtained by feeding a marine type of *Chlorella* which increased intrinsic rate of population and net reproduction rate at 0.88 and 20.0, respectively (Hirayama et al. 1979). Hence, the establishment of the complete nutritional requirement of the rotifer still remains to be studied.

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Table 1. Indices r and Ro obtained from individual culture method for Vitamins A, D and E.

Nutrients added to basic food suspension*	Concentration (µg/ml)	r		Ro	
		Experiment 1	Experiment 2	Experiment 1	Experiment 2
Vit. A	0	0.273	0.386	5.15	6.07
	2.0		0.438		9.82
	5.0	0.288		6.67	
	10.0		0.397		7.11
	20.0	0.188		3.10	
Vit. D	0	0.323	0.293	3.84	4.66
	0.1		0.353		6.78
	0.2	0.424		6.27	
	0.4		0.361		7.66
	0.8	0.314		3.39	
Vit. E	0	0.356	0.399	4.99	6.55
	0.5		0.460		8.36
	1.0	0.429		8.10	
	2.0		0.424		7.09
	4.0	0.361		6.29	

*The nutrients were added to the basic food suspension consisting of 200 µg/ml of baker's yeast and 1.4 µg/ml of Vit. B_{12}.

Table 2. Indices r and Ro obtained from the combination of vitamins A, D and E by individual culture method.

Concentrations of added vitamins in µg/ml*				
A	D	E	r	Ro
0	0	0	0.167	2.7
2.0	0	0	0.307	5.49
1.0	0.1	0.5	0.348	7.0
2.0	0.2	1.0	0.399	8.33

*The vitamins were added to the basic food suspension consisting of 200 µg/ml of baker's yeast and 1.4 µg/ml of Vit. B_{12}.

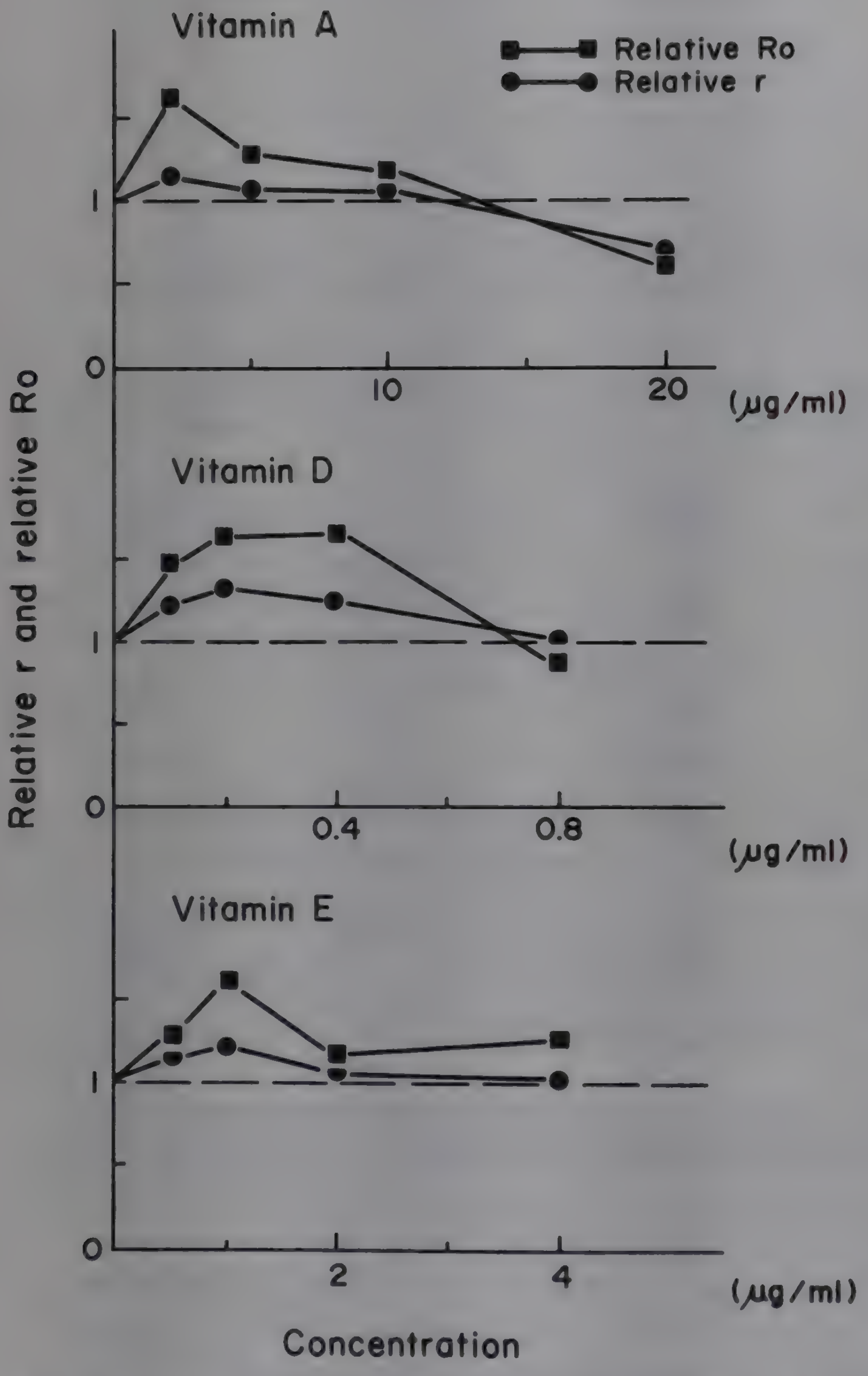

Fig. 1. Relative r and relative Ro of each vitamin at different concentrations.

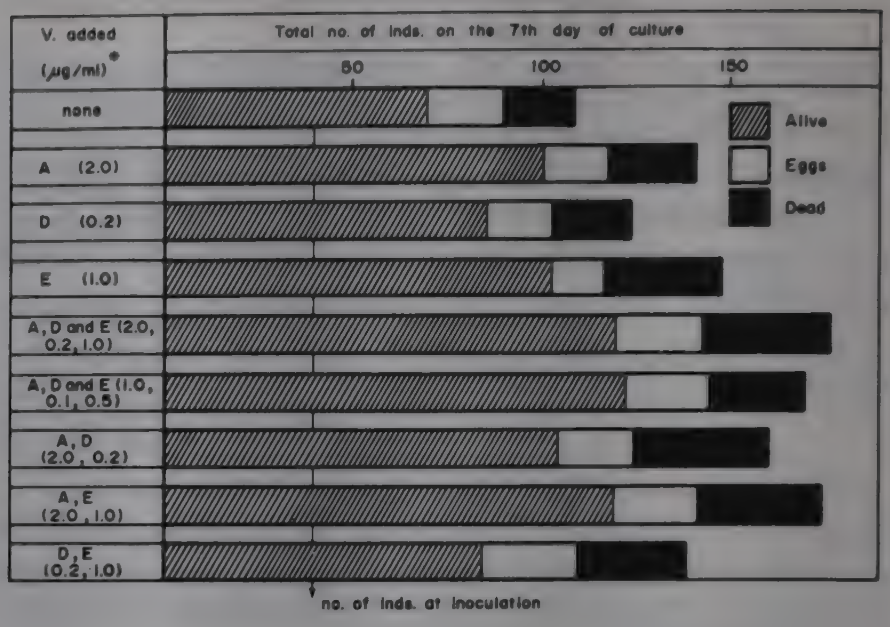

Fig. 2. Total increase in population of rotifer in food suspensions supplemented with the vitamins at different combinations using batch culture method.

*The vitamins were added to the basic food suspension consisting of 200 μg/ml of baker's yeast and 1.4 μg/ml of Vit. B_{12}.

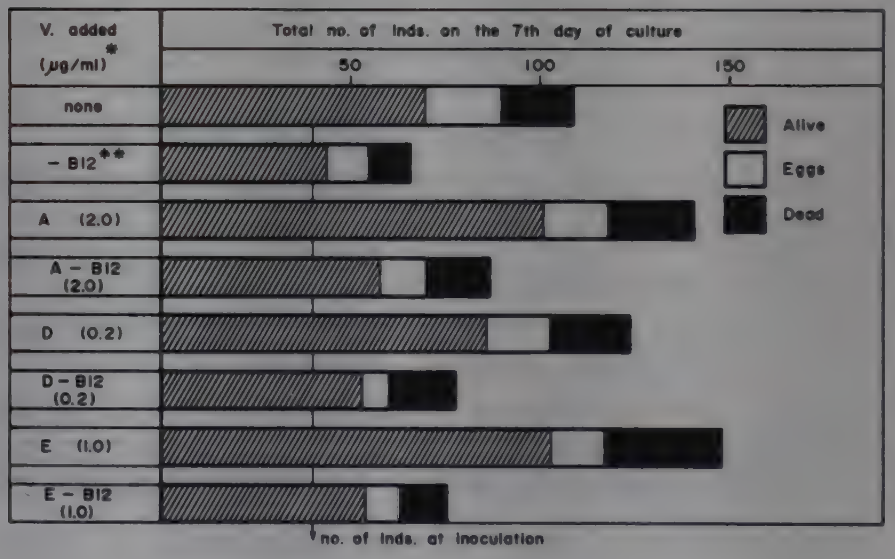

Fig. 3. Total increase in population of rotifer in food suspensions with and without vitamin B_{12} using batch culture method.

*The vitamins were added to the basic food suspension consisting of 200 μg/ml of baker's yeast and 1.4 μg/ml of Vit. B_{12}.

**Vitamin B_{12} was removed from the basic food suspension.

Standard and Routine Metabolic Rate, Critical Oxygen Tension and Spontaneous Scope for Activity of Tilapias

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Abstract

It was the aim of the present study to do comparative tests with four tilapia species and four hybrids under identical conditions on the level of standard, routine and active metabolism to get their specific requirements in different environments. The respiration measurements were carried out with the help of fully automatic computer-regulated equipment, enabling continuous 24-hour measurements of the oxygen intake in up to 16 respirometers. The experiments were done at the Institute of Zoology of Tel Aviv University, Israel, using electrophoretically clearly identified fishes. With the exception of *O. aureus* x *S. galilaeus* F_1, the standard metabolic rates (SMR) of all hybrids tended to be higher than those of the pure-bred species. Apart from *O. niloticus* x *S. galilaeus* the routine metabolic rate (RMR) was also on a higher level. The clearly diverging scope for spontaneous activity (SSA) in red tilapia is of interest in this context. Further, the metabolic reactions were investigated at higher water temperatures (30, 35°C), exceeding the optimum temperature range. The temperature effects on "handling stress" and the critical oxygen point (P_c) are also discussed.

Introduction

Optimizing production in various aquaculture systems will hardly be possible without a good understanding of the interdependence between temperature, oxygen pressure (PO_2), critical O_2-tension (P_c) and oxygen consumption (VO_2) of important cultivated fish. Although some papers have been published dealing with the influence of temperature (Yashouv 1959; Job 1969a; Ross and Ross 1983) and PO_2 (Farmer and Beamish 1969; Caulton 1982; Ross and Ross 1983) on tilapia's VO_2, such essential parameters as standard metabolic rate (SMR), scope for spontaneous activity (SSA) and P_c are still missing. This paper deals with the measurement of all such parameters in relation to water temperature through comparison of their effects on four pure-bred species *(Oreochromis niloticus, O. aureus, Sarotherodon galilaeus, Tilapia zillii)* and four hybrids *(O. niloticus* x *S. galilaeus, O. aureus* x *S. galilaeus* F_2, *O. niloticus* x *O. mossambicus)* all of which are widespread in the tropics and subtropics.

Materials and Methods

The experiments were carried out in the Department of Zoology of the University of Tel-Aviv in Israel on electrophoretically-identified species, some of which had been kept for years in the Institute's aquaria. The experiments took place between November 1983 and October 1984 in a blind room. When respiration was being measured, a dark/light cycle of 12 hours each was adopted. Slight fluctuations often occurred as each new temperature level was reached. If no respiration experiments were taking place, the fish were kept in 300 to 500-l glass aquaria. Restrictive feeding (maintenance level) was carried out with protein and energy of plant and animal origin. The last feed always took place 48 hours before starting experiments. Therapeutic and prophylactic steps were unnecessary as there were no signs of illness during the long period of experimentation.

The recirculating system with a total capacity of 350 l consisted of pump vessel, water reservoir (overflow tank) and four respirometers made of plexiglass in rectangular form with a volume of 12.4 l each. The circulating water was substituted with new water at a rate of 0.2 l/min (290 l/day).

A thermostat regulated temperatures (precision ± 0.2°C). PO_2 was measured constantly with an O_2 electrode (Wissenschaftliche Werke Weilheim) and analyzed by computer. The fully-automatic data collecting and analysis of up to 16 respirometers at one time have been recorded and described in full by Becker (1984) and Sondermann et al. 1985.

All fish were acclimatized for several months at 26°C and their energy metabolism measured. Measurements also took place at 30°C for *O. niloticus, O.*

aureus, *S. galilaeus* and *T. zillii*. Each new test temperature was reached in steps of 0.5°C day. Respiration measurements were carried out 3-4 weeks later, lasting at least 120 hours and sometimes even 240 hours in each cage.

The standard rate of O_2 consumption (SMR) was determined when the fish had grown used to the respiration chamber approximately after 24 hours. Then the VO_2 values collected at the start of the experiments were tested for SMR which was attained when low metabolic rates had been measured constantly over 60 min. (±15% divergence from mean value). Next, the fish were adapted to the new test conditions outside the respiration chamber and the corresponding base metabolic rate re-determined irrespective of water temperature. The PO_2 was > 180 mbar (> 18 kPa) irrespective of water temperature.

Routine VO_2 or RMR is the O_2-consumption of a fasting fish over 24 hours including the VO_2 resulting from spontaneous activity. Average values were obtained irrespective of test temperature from over 1,500 individual figures. Respiratory data for 24 hours were derived from 96 samples taken at fixed times. A fish' VO_2 is correlated directly to its spontaneous activity (SSA) which increases with rising temperatures to a critical level and then declines. Mathematically, the corresponding basic values (SMR) were subtracted from the highest VO_2 values recorded in the first 48 hours of experiment.

The critical O_2 tension point (P_c) was reached when VO_2 (SMR) becomes dependent on PO_2. With the flow rate through the respiration chambers kept constant, a continuous reduction in PO_2 was reached by the fish themselves through their breathing activities in the hermetically-sealed system. This technique leads to a steady reduction in O_2-concentration and enabled the fish to adapt to low PO_2 values (usually > 24 hours). Oxygen uptake independent of PO_2 was assumed until the average SMR could be maintained no longer. Unfortunately, it was not possible to determine the P_c for all species and water temperatures.

All values measured were tested with mathematical models (BUDP) according to Dixon (1985). After completing a variance analysis of the factors' effects on VO_2 the species' mean values were compared in a t-test ($P < 0.05$).

Results

All fish remained in good condition throughout the extremely long experiment. No losses were recorded. As temperature rose, fish showed preference for food of animal origin. At temperatures of 30-35°C, plant food was hardly accepted. All essential test data have been compiled in Table 1.

The SMR, RMR and SSA of all fish tested in the various temperature phases are assembled in Tables 2 and 3. Each metabolic rate was related not only to the absolute body weight but also to the metabolic body mass ($W^{0.8}$). At the acclimatization temperature level of 26°C, the hybrid *O. niloticus* x *S. galilaeus* F_2 also attained a higher SMR than *O. niloticus*. However, at 30°C, *O. niloticus* had overtaken all other species and *T. zillii* was left far behind (Table 3). Metabolic rate of *T. zillii* definitely rises at 35°C although this VO_2 is still very much lower than the SMR of *O. niloticus* and *S. galilaeus*.

The VO_2 routine rate (RMR) in Tables 2 and 3 was parallel to SMR. Any spontaneous activity was included in the RMR. In a species comparison at 26°C, SMR was on average 30% lower than RMR - ranging from -14% *(O. niloticus* x *S. galilaeus)* to -49% *(O. aureus* x *S. galilaeus)* (relative divergence). As temperatures rose, SMR and RMR gradually drew together so that the divergence shrunk to only -17% when a temperature of 35°C was reached. This applied especially to *O. niloticus* and *S. galilaeus*.

Tables 2 and 3 include data on the aerobic scope for spontaneous activity (SSA) which can be calculated by subtracting the SMR from the maximum VO_2 recorded. The hybrids of *O. niloticus* and *O. mossambicus* had the highest spontaneous O_2 consumption of all species. *O. aureus* x *S. galilaeus* also differ considerably from the others, statistically speaking, with the exception of the F_2 generation of *O. niloticus* only. Of special interest was the reduction in spontaneous VO_2 difference (SSA-SMR) in *O. aureus* when temperatures rose. This can be explained by the significant rise in SMR which occurs simultaneously.

The critical O_2-point (P_c) could only be determined for *O. niloticus* x *O. mossambicus* at the adaptation temperature of 26°C and for *O. niloticus* at 35°C. The corresponding P_c values were 42 and 35 mbar respectively. Unfortunately, some temperature levels are missing for these tests so that an all-round comparison is not possible at this point. Although PO_2 fell to 31 mbar at 26°C and 18 mbar at 30°C, we could not determine any dependence of VO_2 on PO_2 for *T. zillii*. *S. galilaeus* was also able to maintain its SMR at 30°C although PO_2 sank to 35 mbar. The so-called "handling stress" is often used as a gauge for measuring the excitability of fish. It can be plotted in relative values against time as shown in Fig. 1. The ordinate represents the relative rise in metabolic rate (VO_2) in relation to the corresponding SMR after 24 hours. The abscissa shows the exact times the O_2 data were collected. The hyperactive phase was over very quickly: for *O. aureus* after 30 min. and for all other species after one hour. This clear stage of pacification continued for about two hours after the start of the experiment, declining in a linear fashion, then fell at a much lower rate without, however, reaching the reference value (SMR) in the following hours.

Discussion

Using computers to continually register VO_2 in a flow-through system for a long period proved very helpful in eliminating grave problems in measuring respiratory activities. It was possible to pinpoint the exact times at which fish reached their lowest metabolic rate (SMR) by dividing the day into 96 phases of 15 min. each. Thus, phases of spontaneous VO_2 were not included in the SMR. This explains why these experiments revealed a VO_2 for *O. niloticus* of only 74% of the value which Farmer and Beamish published in 1969 although water temperatures were comparable.

If one accepts the continuous VO_2 balance as a sufficiently exact approximation for metabolism, it is possible to reckon the heat production (ME) using the following equation:

$$T\ (kJ) = VO_2\ (g) \times Q_{ox}\ (kJ/g)$$

when an oxienergetic equivalent (Q_{ox}) of 13.78 kJ/g VO_2 in unfed fish is assumed. With data in Table 2, an average energy release of 19.2 ± 3.5 or 17.9 ± 2.5 kJ $kg^{-0.8}$/day when *O. niloticus* x *O. mossambicus* are not considered, can be obtained. In comparison, metabolic rates of 30.4 kJ $kg^{-0.8}$/day were derived for *Cyprinus carpio* at 26°C (Ott et al. 1980; Becker 1984).

When water temperatures rose, tilapia showed a much lower rise in metabolism (Table 3) than carp, reaching only 47% of the SMR of *C. carpio* at 30°C (Ott et al. 1980; Becker 1984), proof of excellent adaptation to water temperature. The important ecological advantage resulting simultaneously is the substantial rise in spontaneous VO_2 (Table 3) for warm-adapted fish. A number of tests for RMR described in publications are listed in Table 4 with findings of their experiments. All metabolic figures were converted to mg O_2 $kg^{-0.8}$/hour showing clearly that VO_2 stands not in an isometric, but in an allometric relationship ($W^{0.8}$) to fish body mass. If Ross and Ross' (1983) extremely high values for the routine metabolic rate of 10-g *O. niloticus* are excluded, it can be said that fasting tilapia need 72.8 ± 14 mg O_2 $kg^{-0.8}$/hour to maintain vital body functions. This average result is almost identical to the mean value of 82.8 ± 14.9 mg O_2 $kg^{-0.8}$/hour obtained in these experiments. Suprisingly, water temperature has very little influence on routine VO_2 (Tables 2 and 3). Ross and Ross (1983) obtained a value of 2, 3 for *O. niloticus* in the same temperature range. Hughes et al. (1983) found a clear dependence of RMR on PO_2 (Q_{10} 1.8-2.4) for carps. The mean Q_{10} value for 26-35°C is 1.46. The amount of spontaneous maximum VO_2 is important not only as regards respiratory activities, but also as a possible parameter in the aquaculture of the future. No comparative values - not even individual results - could be found in existing literature.

The species comparison in Table 3 shows a vast superiority for *O. aureus* x *S. galilaeus* and *O. niloticus* x *O. mossambicus* on the acclimatization temperature 26°C compared to all other species tested. Distinct variations in change of metabolic rate (Table 3) can be found between different temperatures within species.

Total body analyses of tilapia have seldom been carried out (Caulton 1982; Meske et al. 1983) and information on how ME converts to protein and fat energy is non-existent. Extensive experiments with carp (Becker 1984; Koch 1984; Muller 1986) revealed an average efficiency for protein synthesis of 0.52. For the synthesis of body fat the average efficiency is 0.75-0.90. However, calculating with the factor 0.9 is only realistic when a diet rich in energy, i.e., high fat content is offered. If the body lipids are mainly synthesized from carbohydrates then the efficiency is very probably around 0.75. Our model calculation is based on a content of 15% crude protein and 8% crude fat in live fish body mass. The kf-value is 0.9 and the kp-value 0.52. Assuming an energy concentration of 23.65 kJ/g protein and 36.24 kJ/g for fat (Brett and Groves 1979), the energy released as heat/g increase in body mass can be deducted. A heat liberation per gram of body mass increase of 3.9-4.8 kJ seems to be realistic. However, since a maximum efficiency for protein synthesis of 0.87 is theoretically possible (Armstrong 1969), considerably less oxygen per gram body mass increase is feasible. Experiments for measuring the efficiency in carp revealed kp-values ranging from 0.34 to 0.76 (Becker 1984) with a constant kf-value of 0.75.

Aerobic scope for spontaneous activity VO_2 (Tables 2 and 3) can be converted into energy units by means of an oxienergetic equivalent. Growth (protein and fat retention) incurs heat liberation of 14.84 kJ/g O_2 (Brody 1945; Kleiber 1967). If one then multiplies the VO_2 (SSA) which surpasses the SMR in 24 hours by the Q_{ox}, the resulting heat is theoretically the amount of oxygen used in body tissue synthesis. Dividing this value by the energy liberated per gram-growth (3.87 kJ/g) reveals absolute values. The growth rates in Figs. 2 and 3 were obtained in this fashion. If one assumes that these amounts of energy ($VO_2 \times Q_{ox}$) are released when body fat and protein are synthesized then the growth rates deducted in Figs. 2 and 3 are theoretically possible.

For the species comparison, the relation per kg body weight and $W^{0.8}$ (Fig. 2) was chosen. Crosses of *O. aureus* x *S. galilaeus* F_1 and F_2 and *O. niloticus* x *O. mossambicus* differ greatly from the other species in their theoretic growth potential. It remains to be seen whether the latter hybrid can maintain its SSA-won superiority under production conditions. Practical results in intensive aquaculture (Zohar et al. 1986) showed a daily growth of 10.2-11.7 g $kg^{0.8}$/day for *O. aureus* x *O. niloticus* hybrids (all male) in the 140-380 g weight range. As both temperature and average fish mass are similar to those of these experiments, it seems legitimate to state that SSA is a good indicator of genetically, determined growth potential. This potential, however, seems to depend largely on water temperature and species (Fig. 3) as *O. niloticus* and *S. galilaeus* reached their highest prospective growth only at 35°C, whereas *O. aureus* obtained the same effect at 30°C and *T. zillii* at 26°C.

The preliminary character of the P_c values was emphasized already in the paragraphs describing the results of this study. The data were obtained from fasting, carefully-adapted fish at a low stage of metabolism and refer to the SMR recorded at the various temperature levels. In contrast to Ross and Ross (1983) and Ahmed and Magid (1969) who used closed vessels, the system has the advantage of being able to keep the fish on a certain PO_2 level for a fixed period of time as in Ott et al. (1980) and Ultsch et al. (1980). Meaningful results can be expected only after sufficiently long periods of adaptation to steadily-decreasing partial O_2 pressure values as this is the only guarantee for the correctness of the measured SMR (Ultsch et al. 1980). Ross and Ross (1983) discovered in *O. niloticus* a dependence of VO_2 on PO_2 at 30°C when the latter lay between 60 and 80 mbar. One deduces from the authors' statements that, depending on the fish mass which varied between 6.5 and 50 g, the P_c was reached in a closed respirometer (volume 450 ml) after 5.9 and 13 min., respectively. It is hardly conceivable that such a rapid oxygen depletion will occur under more practical conditions.

Present findings revealed a P_c of 42 ± 7.4 mbar for *O. niloticus* x *O. mossambicus* at 26°C and of 35 mbar for *O. niloticus* at 35°C. We could ascertain no reduction in SMR for *T. zillii* even at a P_c of 18 mbar (30°C). These values and the lack of P_c's dependency on water temperature coincide with the results obtained by Ott et al. (1980) and Ultsch et al. (1980).

Of special interest is the observation, in this study, of a new stable - although lower - SMR in fish which could have reached their critical O_2 level, if the PO_2 prevailing at that point was kept constant. Such reactions are regulated by an increase in hemoglobin concentration from accumulated reserves and an intensified blood circulation as well as the opening of additional capillaries in gills and tissue areas (Prosser et al. 1957; Heisler, pers. comm.).

Handling fish (e.g., determining mass and transferring fish from large aquaria to respiratory chambers) leads to a short-lived rise in oxygen consumption. *T. zillii* proved to be largely insensitive but other species regained their basic values rapidly, taking only 6-12 hours. Such results must be interpreted with great care as far as the individual species' capacity for enduring stress is concerned because Farmer and Beamish (1969) discovered that adaptive reactions can significantly lower O_2 uptake when stress is induced repeatedly. For this reason comparing these parameters for different temperature levels in one and the same fish cannot be recommended.

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Table 1. Experimental characteristics: species, body mass and temperature levels.

Species	n	Average body mass		Test temperature	Parameters tested			
		g	s.d	°C	SMR	RMR	SSA	P_c
Oreochromis niloticus	4	310	34	26	+	+	+	–
				30	+	+	+	+
				35	+	+	+	+
Sarotherodon galilaeus	4	283	47	26	+	+	+	–
				30	+	+	+	+
				35	+	+	+	–
Oreochromis aureus	4	687	287	26	+	+	+	+
				30	+	+	+	–
				35	+	+	+	–
Tilapia zillii	2	315	130	26	+	+	+	+
				30	+	+	+	+
				35	+	+	+	–
Oreochromis aureus x *Sarotherodon galilaeus* F_1	4	467	65	26	+	+	+	–
Oreochromis aureus x *Sarotherodon galilaeus* F_2	2	309	–	26	+	+	+	–
Oreochromis niloticus x *Sarotherodon galilaeus*	3	700	183	26	+	+	+	+
Oreochromis niloticus x *Oreochromis mossambicus*	4	286	60	26	+	+	+	+

Table 2. Metabolic rates (VO_2; means ± s.d.) of different species and hybrids of tilapia at 26°C

Species	Standard metabolic rate		Routine metabolic rate		Aerobic scope for spontaneous activity	
	mg/kg/hr	mg/kg$^{0.8}$/hr	mg/kg/hr	mg/kg$^{0.8}$/hr	mg/kg/hr	mg/kg$^{0.8}$/hr
Oreochromis niloticus	56.9 ± 6.6^{a}	44.4 ± 4.5^{a}	94.0 ± 12.1^{a}	73.8 ± 8.6^{a}	124.3 ± 24.9^{a}	97.7 ± 18.6^{a}
Sarotherodon galilaeus	69.3 ± 10.3^{a,b}	53.6 ± 6.5^{a,b}	109.7 ± 13.5^{a,b}	84.9 ± 9.3^{a,b}	132.8 ± 37.0^{a,b}	102.5 ± 25.8^{a,b}
Oreochromis aureus	65.4 ± 10.4^{a,b,c}	59.9 ± 6.7^{b,c}	79.0 ± 17.7^{a,c}	72.2 ± 13.0^{a,b,c}	103.9 ± 37.3^{a,b,c}	94.3 ± 29.4^{a,b,c}
Tilapia zillii	58.6 ± 12.2^{a,b,c,d}	48.5 ± 9.5^{a,b,c,d}	77.5 ± 10.2^{a,c,d}	64.4 ± 7.7^{a,c,d}	134.0 ± 26.5^{a,b,c,d}	111.2 ± 20.8^{a,b,c,d}
Oreochromis aureus x *Sarotherodon galilaeus*	54.9 ± 5.6^{a,b,c,d,e}	47.5 ± 5.3^{a,b,d,e}	106.9 ± 4.6^{a,b,d,e}	92.5 ± 3.9^{b,e}	223.7 ± 72.4^{e}	192.8 ± 58.6^{e}
Oreochromis niloticus x *Sarotherodon galilaeus*	67.4 ± 11.5^{a,b,c,d,e,f}	62.3 ± 7.7^{b,c,d,f}	78.3 ± 11.8^{a,c,d,f}	72.4 ± 8.2^{a,b,c,d,f}	113.5 ± 3.7^{a,b,c,d,f}	105.2 ± 4.2^{a,b,c,d,f}
Oreochromis aureus x *Sarotherodon galilaeus* (F_2)	80.1 ± 26.1^{b,c,d,f,g}	63.2 ± 19.8^{b,c,d,f,g}	118.5 ± 26.6^{a,b,e,g}	93.5 ± 19.7^{a,b,e,f,g}	164.8 ± 3.3^{a,b,c,d,e,f,g}	122.2 ± 1.0^{a,b,c,d,f,g}
Oreochromis mossambicus	110.8 ± 14.8^{h}	84.2 ± 6.9^{h}	144.4 ± 31.1^{a,h}	109.3 ± 16.4^{a,h}	388.0 ± 46.5^{h}	296.6 ± 42.8^{h}

Table 3. Metabolic rates (VO_2; means ± s.d.) at varying temperatures within species.

Species		Standard metabolic rate		Routine metabolic rate		Aerobic scope for spontaneous activity	
		mg/kg/hr	mg/kg$^{0.8}$/hr	mg/kg/hr	mg/kg$^{0.8}$/hr	mg/kg/hr	mg/kg$^{0.8}$/hr
Oreochromis niloticus	26°C	56.9 ± 6.6^{a}	44.4 ± 4.5^{a}	94.0 ± 12.1^{a}	73.8 ± 8.6^{a}	124.3 ± 24.9^{a}	97.7 ± 18.6^{a}
	30°C	111.4 ± 13.7^{b}	88.4 ± 9.5^{b}	142.8 ± 13.0^{b}	113.3 ± 8.6^{b}	135.5 ± 10.1^{a,b}	107.6 ± 8.2^{a,b}
	35°C	118.2 ± 8.7^{b,c}	93.8 ± 6.4^{b,c}	134.6 ± 8.9^{b,c}	106.7 ± 6.1^{b,c}	249.3 ± 86.1^{c}	196.9 ± 64.5^{c}
Sarotherodon galilaeus	26°C	69.3 ± 10.3^{a}	53.6 ± 6.5^{a}	109.7 ± 13.5^{a}	84.9 ± 9.3^{a}	132.8 ± 37.0^{a}	102.5 ± 25.8^{a}
	30°C	80.5 ± 8.5^{a,b}	62.5 ± 5.8^{a,b}	121.1 ± 17.5^{a,b}	94.1 ± 12.1^{a,b}	251.8 ± 165.0^{a,b}	195.6 ± 127.7^{a,b}
	35°C	133.6 ± 11.2^{c}	103.2 ± 6.0^{c}	157.3 ± 15.4^{c}	121.4 ± 8.3^{c}	269.7 ± 110.4^{a,b,c}	206.4 ± 76.5^{a,b,c}
Oreochromis aureus	26°C	65.4 ± 10.4^{a}	59.9 ± 6.7^{a}	79.0 ± 17.7^{a}	72.2 ± 13.0^{a}	103.9 ± 37.3^{a}	94.3 ± 29.4^{a}
	30°C	65.4 ± 10.1^{a,b}	60.7 ± 8.8^{a,b}	106.7 ± 22.3^{a,b}	98.4 ± 16.3^{b}	148.6 ± 28.5^{a,b}	137.0 ± 19.7^{b}
	35°C	91.8 ± 17.9^{c}	83.7 ± 14.1^{c}	117.3 ± 21.6^{b,c}	106.9 ± 15.9^{b,c}	108.2 ± 34.0^{a,b,c}	98.4 ± 26.1^{a,b,c}
Tilapia zillii	26°C	58.6 ± 12.2^{a}	48.5 ± 9.5^{a}	77.5 ± 10.2^{a}	64.4 ± 7.7^{a}	134.0 ± 26.5^{a}	111.2 ± 20.8^{a}
	30°C	43.4 ± 3.6^{a,b}	35.7 ± 3.4^{a,b}	80.1 ± 6.9^{a,b}	65.8 ± 4.7^{a,b}	93.5 ± 19.1^{a,b}	76.7 ± 14.7^{a,b}
	35°C	81.4 ± 3.8^{a,c}	66.6 ± 2.4^{a,c}	99.7 ± 4.2^{a,b,c}	81.6 ± 2.6^{a,b,c}	110.9 ± 12.7^{a,b,c}	90.6 ± 9.3^{a,b,c}

Table 4. Data from literature regarding the oxygen consumption of fasting fish.

Species	Masses g	Temp. °C	O_2 consumption, mg kg	O_2 consumption, mg $kg^{-0.8}$	References
Oreochromis niloticus	10	26	1,174	487	Ross and Ross 1983
	50	25	133	73	Ross and Ross 1983
mg/kg/hr	80	25	100*	60*	Farmer and Beamish 1969
	143	?	81	62	Ahmed and Magid 1969
	330	?	102	82	Ahmed and Magid 1969
Oreochromis mossambicus	90	30	262	78	Job 1969b
	10	25	239	96	Caulton 1982
	80	25	115	69	Caulton 1982
	150	26	92	63	Caulton 1982
Oreochromis niloticus	310	26	81	74	own data
Oreochromis aureus	697	26	84	72	own data
Sarotherodon galilaeus	283	26	107	86	own data
Tilapia zillii	316	26	77	64	own data
Oreochromis niloticus x Sarotherodon galilaeus	700	26	78	72	qwn data
Oreochromis aureus x Sarotherodon galilaeus	487	26	103	93	own data (F_1)
Oreochromis niloticus x Oreochromis mossambicus	266	26	144	109	own data
Oreochromis aureus x Sarotherodon galilaeus	309	26	114	90	own data (F_2)
*SMR			x 120.1	x 77.8	
			± 54.4	± 14.0	
			VC 45.3	VC 18.1	

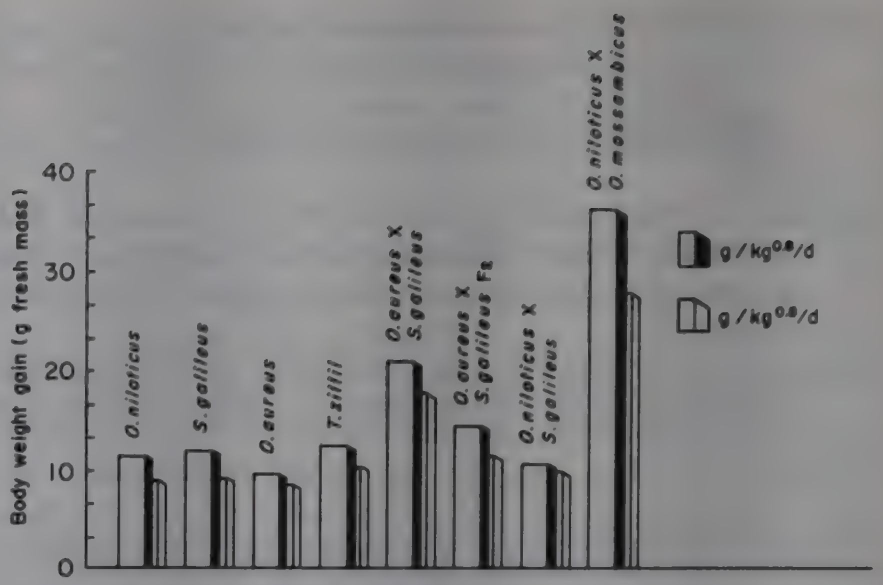

Fig. 2. Potential for growth based on theoretical inference from scope for spontaneous activity (SSA) of different tilapia species at 26°C.

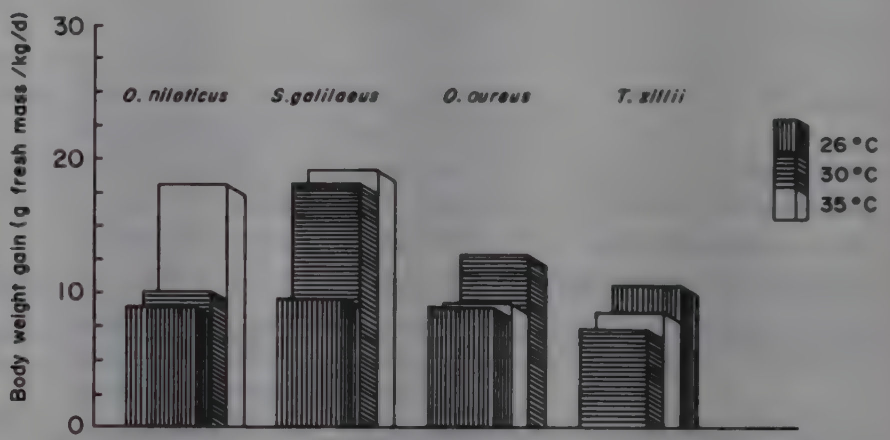

Fig. 3. Potential for growth based on theoretical inference from scope for spontaneous activity (SSA) of different tilapia species and different temperatures.

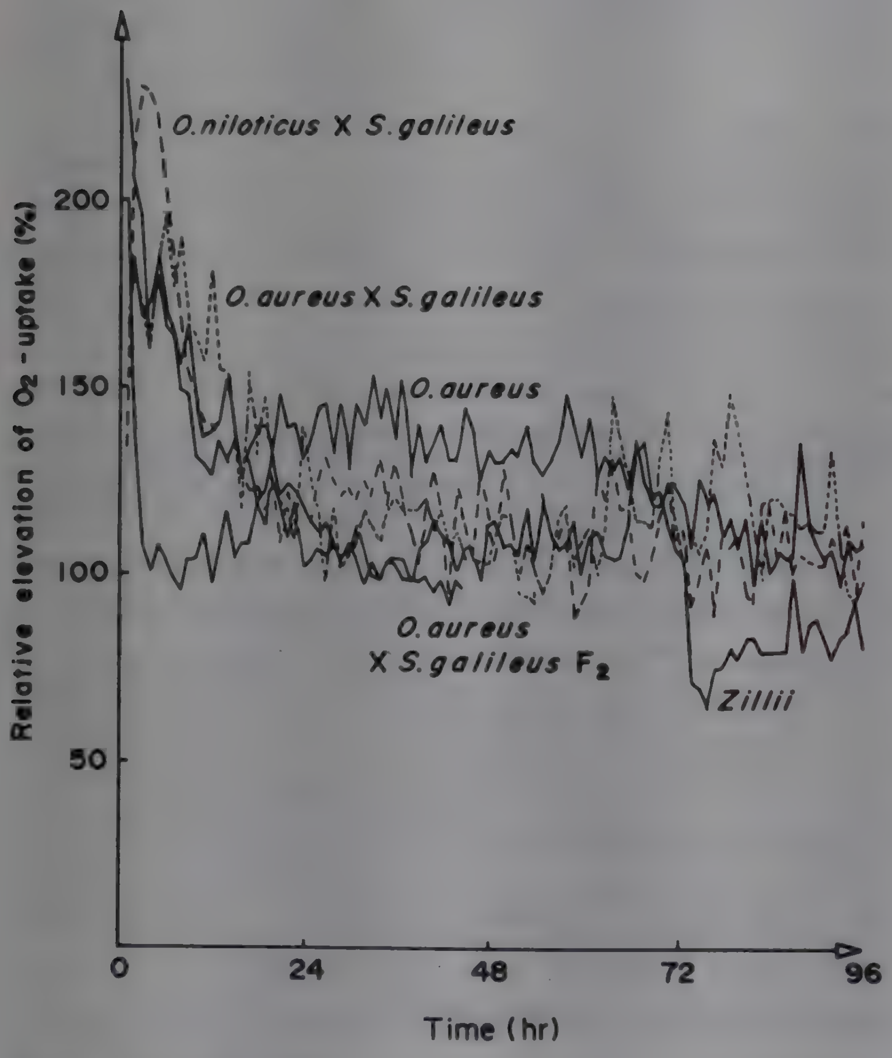

Fig. 1. Stress induced VO_2 increase related to O_2-uptake 24 hours after handling.

Metabolic and Osmotic Responses of *Metapenaeus ensis* (De Haan) Subjected to Sudden Salinity Change

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Abstract

The resting respiration rate of 5- to 7-gram *Metapenaeus ensis* (De Haan) was continuously monitored in an open circulation system in Taiwan. The respiration rate is characterized by the following consecutive metabolic responses to sudden salinity change: a short period of acute increase, a depression, an increase and then restoration of the stable respiration rate. It takes two to three hours to stabilize respiration rate and approximately five hours for internal osmolarity to become stable after the change. The amplitude of the salinity change, and hence the osmotic pressure, affect the range of metabolic responses.

Introduction

The study of respiration rate of crustaceans started in the beginning of the 20th century (Wolvexkamp and Waterman 1960). Until recently, most of the studies were performed in closed systems, i.e., the dissolved oxygen in the test chamber decreased along with the oxygen consumption of the animals. However, the oxygen tension in the water itself influences the respiration rate (McManon et al. 1974; Davis 1975; Taylor 1976). To obtain confident basic metabolic data, the open system should be used to measure respiration rates. In this study, a simple open system allowed continuous data recording, blank measuring and a change of experimental conditions without physical disturbance of the test animal.

The dynamic response of the respiration rate of the shrimp, *Metapenaeus ensis* (De Haan), during the salinity change was studied. Previous studies focused on the metabolic rate of shrimp in different salinities.

Materials and Methods

Shrimp weighing 5.2 to 7.5 g were kept in the laboratory at room temperature for at least one week before acclimation to different salinities. Shrimp in the D-stage of molting were selected for experiments. They were fed with shrimp meat during rearing but were made to fast one day before the experiment.

Fig.1 presents the chamber for measuring respiration rate. Aerated water was pumped either into the experimental chamber or blank chamber. The flow rate was adjusted by the pump (88-110 ml/min.). Shrimp were constrained by a small net of 4-mm^2 mesh size to measure resting respiration rate. Three shrimp were used per test. Usually, it took three hours for the subject to stabilize before respiration rate data were taken.

The salinity of experimental water was adjusted either by adding freshwater or 200 ppt artificial seawater produced by introducing NaCl: MgCl + 6H$_2$O: KCl: CaSO$_4$ (100:16.7:2.7:0.8 w/w) into aged seawater. Final salinity was determined by AgNO$_3$ titration. It took approximately 10-12 min. for a salinity change of 20 ppt in the experimental chamber.

All the experiments were performed in thermo-bath of 24.5-25.5°C.

To measure osmolarity, shrimp were first treated with different salinities for various periods then hemolymph was drawn from the pericardial cavity. A Wiscor 5100 C vapor pressure osmometer was used to measure the osmolarity.

Results

In low the salinity change (< 5 ppt), the fluctuation of the respiration rate was as shown in Fig. 2. A change of 1.17 ppt slightly increased the rate. When the salinity increase was over 2 ppt, the oxygen consumption of the animal increased followed by a depression within 1 min. after change. It went back to normal after several hours. An increase or decrease in salinity aroused similar responses. Note that salinity change alone caused fluctuations on the meter, but always less than 2%.

Figs. 3 and 4 show the influence of high salinity change on respiration rate. A respiration pattern of sudden depression followed by a small increase, then instability for several hours appeared. Shifts to very high salinities, 29.74 to 49.33 ppt, or 40.18 to 49.53 ppt, produced high amplitudes of respiration fluctuation and took more than 4 hours to become stable. Mortality rate was 70% after one week of acclimation.

The respiration rate of *M. ensis* in different salinities from 10 to 50 ppt averaged 140 mg/kg/hour, similar to the resting metabolic rate of 5.5-g *Penaeus japonicus* (around 117 ml/kg/hour). Two-way analysis of variance (ANOVA) showed the difference between each salinity was insignificant.

Fig. 5 indicates the internal osmolarity of the shrimp vs. the osmolarity of the medium after three days of acclimation. The isosmotic point was around 22 ppt salinity.

Fig. 6 plots salinity change vs. respiration rate change for the first 5 min. The response of respiration was very sensitive to the salinity change of the medium. A slight change of 1 ppt caused an observable response. Changes over 2 ppt caused drastic fluctuation but their amplitude did not necessarily correlate with the salinity change.

Fig. 7 shows the steady increase in internal osmolarity of the shrimp after transfer into a high salinity medium.

Discussion

The gill of shrimp not only functions as a respiratory organ but also as a place where ion exchange occurs (Mantel and Farmer 1983). This may be the reason for respiratory depression during salinity change. The animal could have purposely reduced the water flow through the gill to resist the salinity shock, which would reduce oxygen consumption. An increase in respiration rate occurred followed by a period of restoration during which there was short-term fluctuation of the respiration rate. This is because the respiratory water flow of shrimp is produced by scaphognathite activity; the external salinity change will disturb this movement. A similar phenomenon was observed in the crab, *Carcinus maenas*. When it was transfered from 100% seawater to 15% seawater, the scaphognathite activity became slower, and even stopped a few seconds (Hume and Berlind 1976).

M. ensis is a moderate hyper- and hyporegulator (Mantel and Farmer 1983). However, since the metabolic rate of this shrimp is somewhat stable in the range of 10 to 40 ppt, it seems that active osmoregulation did not require much energy. Eltringham (1965) found a similar phenomenon in *Limnoria* sp. in hypotonic medium. Kirkpatrick and Jones (1985) found the osmolarity of *Palaemon affinis* took 72 hours to become stable after a salinity change while the stability of *M. ensis* was achieved five hours after the change. Therefore, *M. ensis* is a typical euryhaline species as defined by Kinne (1971).

There was no significant difference between the respiration rate of male and female individuals although none of the samples had reached mature stage.

Acknowledgements

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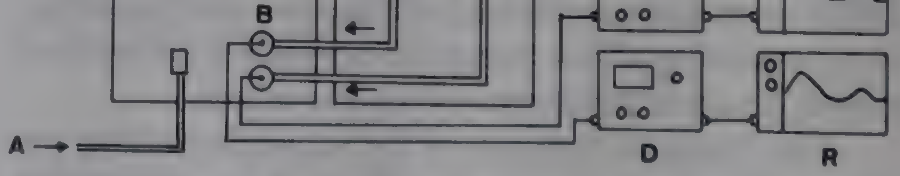

Fig. 1. The device for measuring respiratory rate. A: air, B: DO meter probe, C: water bath tank, D: DO meter, P: pump, R: recorded, T: aeration tank.

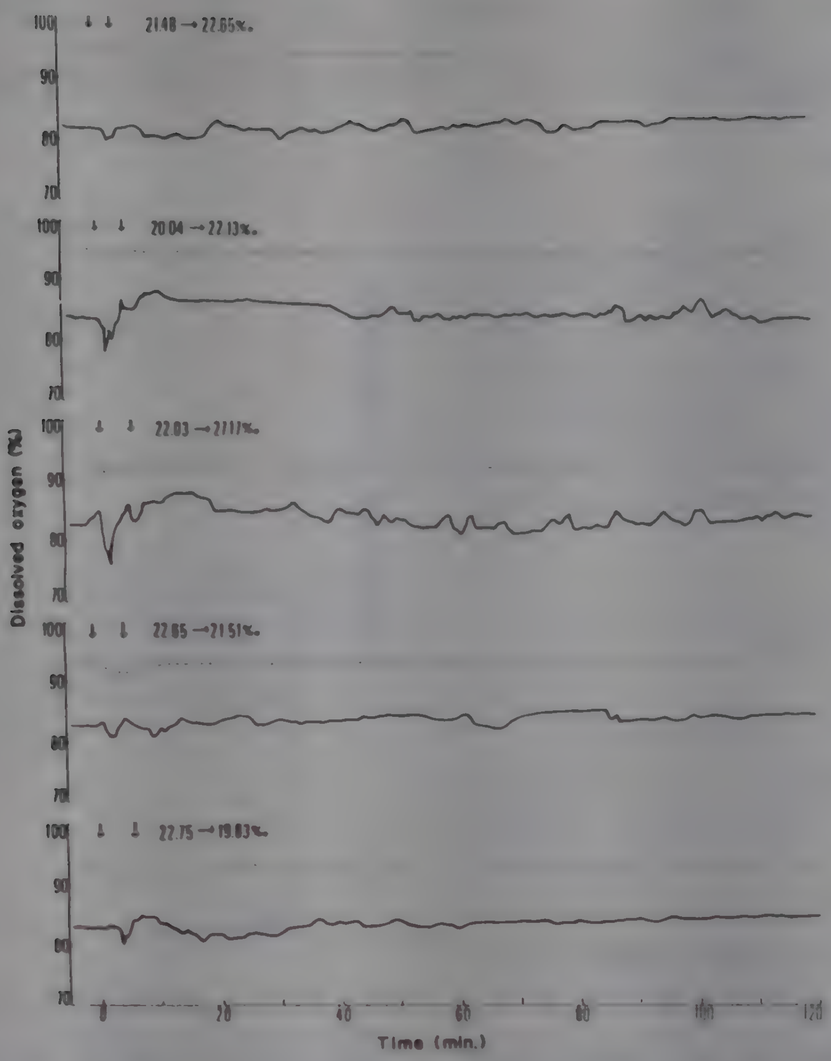

Fig. 2. Effect of small salinity change on the respiration rate of *Metapenaeus ensis*. Broken lines represent the dissolved oxygen of blank chamber. Solid lines depict the dissolved oxygen of respiratory chamber. Arrows indicate start and end of salinity change.

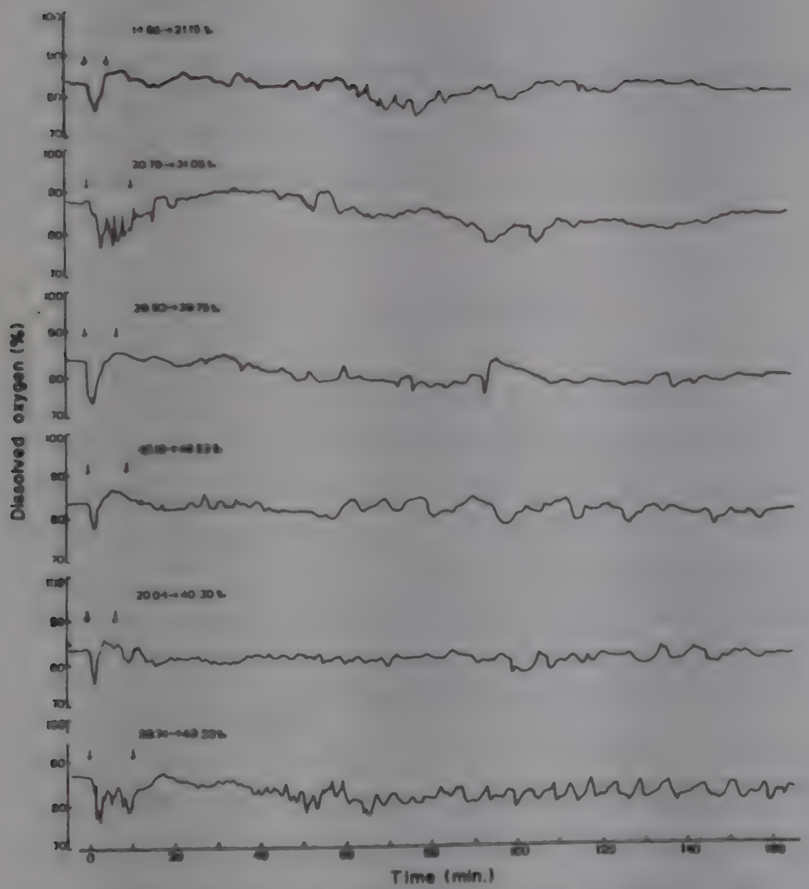

Fig. 3. Effect of large salinity increases on the respiration rate of *Metapenaeus ensis*. Arrows indicate start and end of salinity change.

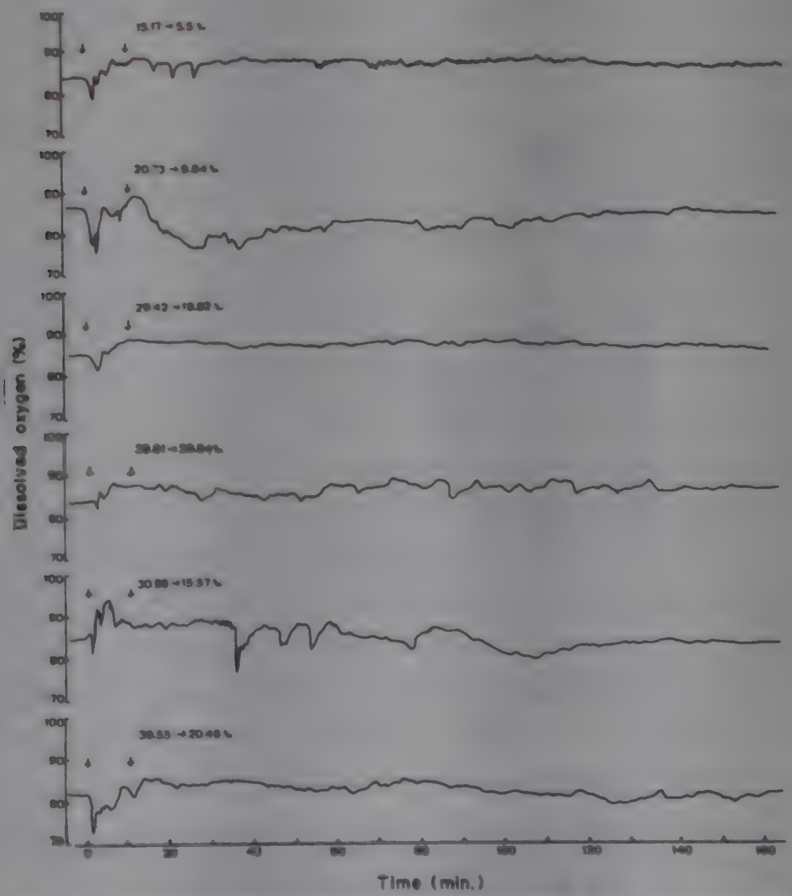

Fig. 4. Effect of large salinity decreases on the respiration rate of *Metapenaeus ensis*. Arrows indicate start and end of salinity change.

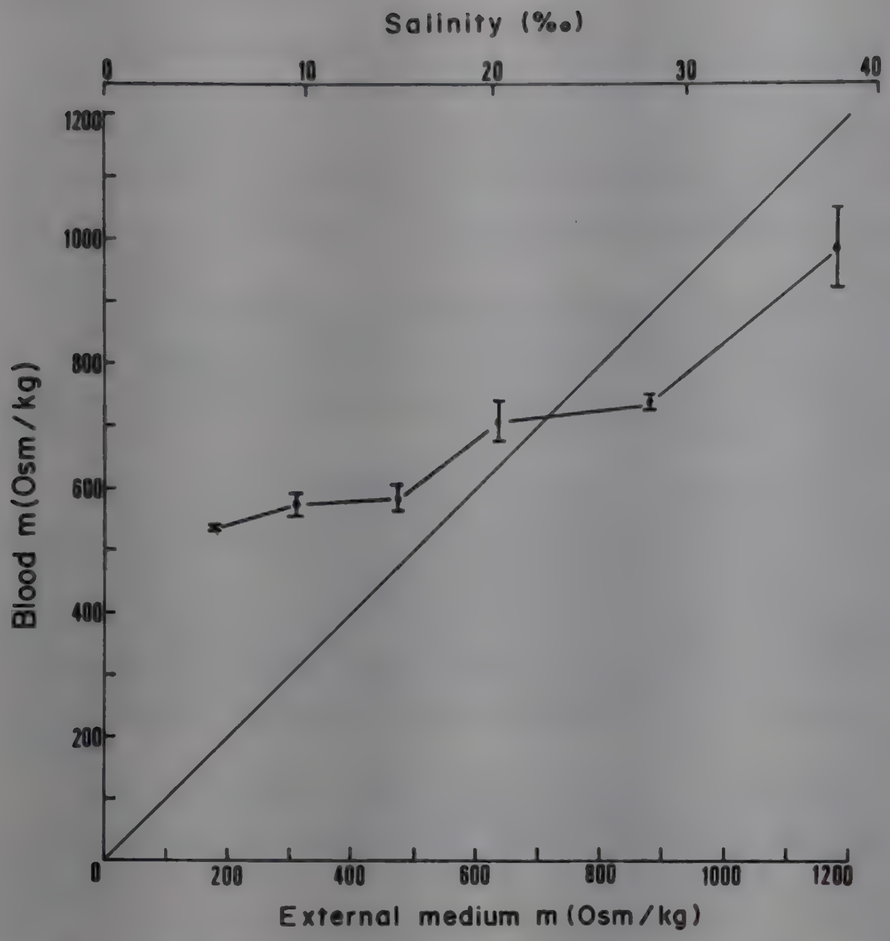

Fig. 5. Blood osmolarity of *Metapenaeus ensis* in media of different salinities. Vertical lines indicate 95% confidence limit.

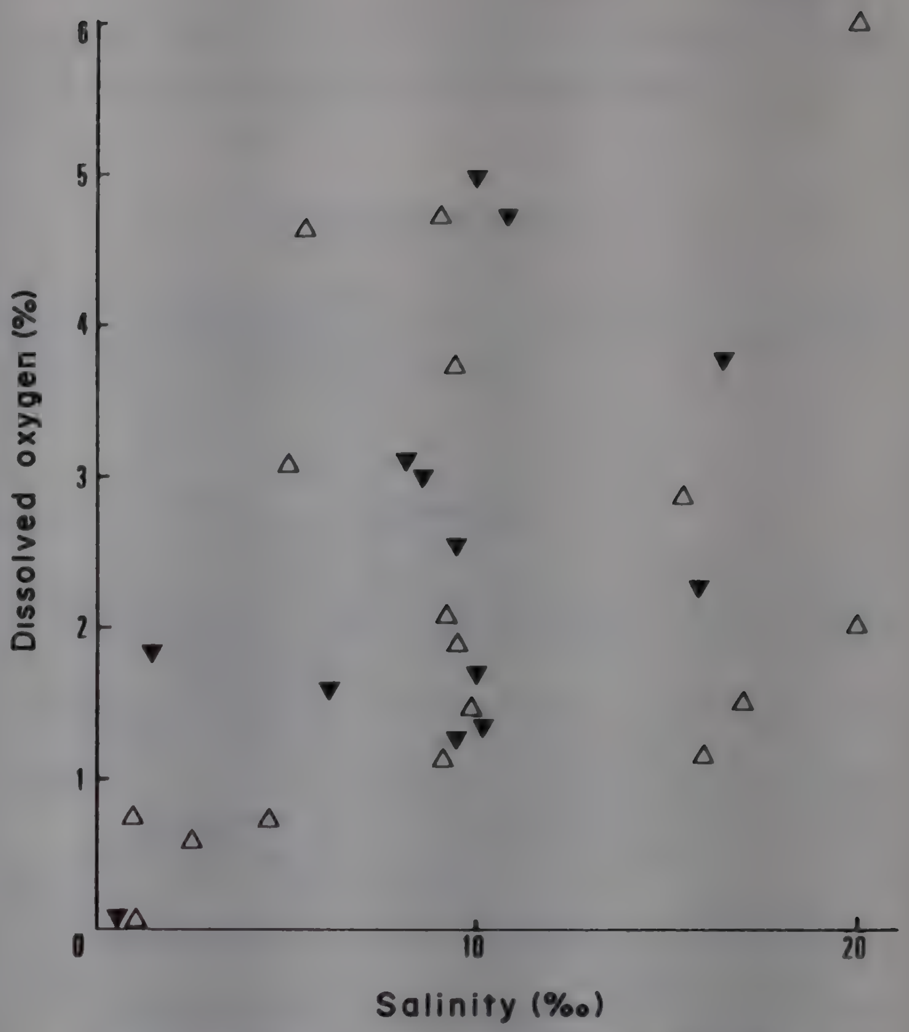

Fig. 6. The influence of salinity change on the short-term (5 min.) oxygen consumption of *Metapenaeus ensis*. △, salinity increase; ▼, salinity decrease.

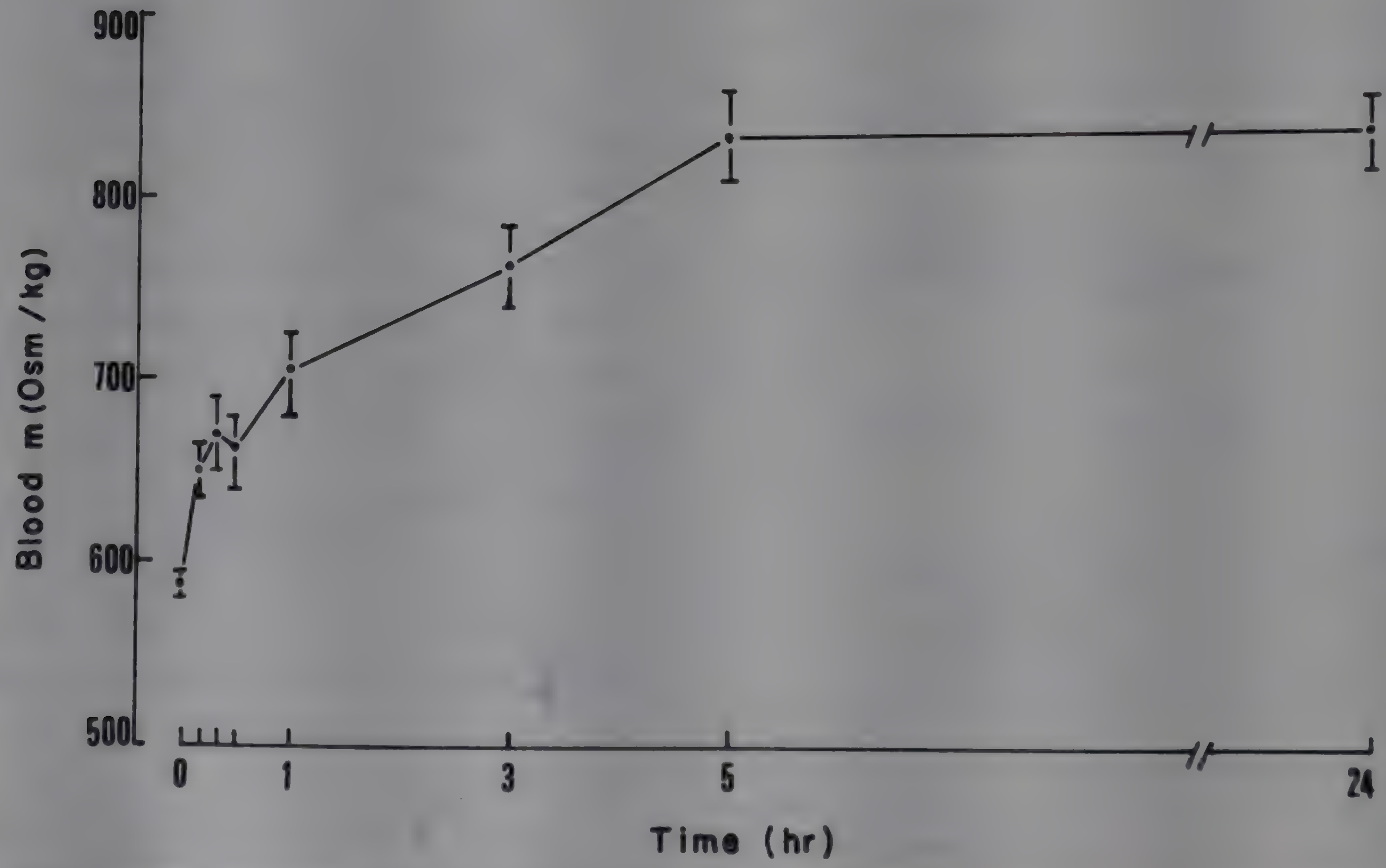

Fig. 7. Blood osmolarity change of *Metapenaeus ensis* after rapid transfer from 17.4 to 33.7 ppt. Vertical lines indicate 95% confidence limit.

The Effect of Salinity on the Osmotic and Ionic Concentrations in the Hemolymph of *Penaeus monodon* and *P. penicillatus*[1]

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Abstract

The osmoregulation of juvenile and adult *Penaeus monodon* and *P. penicillatus* and the ionic regulation of the adults, were investigated over a salinity range of 3-50 ppt. *P. monodon* was found to be a stronger osmotic and ionic regulator than *P. pencillatus*. The juveniles of both species are stronger osmoregulators than adults. The salinity ranges over which osmoregulations were performed efficiently were 3-50 ppt for juvenile *P. monodon*, 15-50 ppt for adult *P. monodon*, 5-30 ppt for juvenile *P. penicillatus* and 15-30 ppt for adult *P. penicillatus*. The isosmotic points were 730, 750, 747 and 800 mOsm/kg, respectively. The curves of sodium, chloride and potassium corresponded to those for the osmotic concentration of adults. The respective isoionic points for *P. monodon* and *P. penicillatus* were 352 mEq/l, 348 mEq/l for sodium; 320 mEq/l, 304 mEq/l for chloride; and 7.1 mEq/l, 6.1 mEq/l for potassium. Calcium was accumulated in both species, while magnesium levels were strongly reduced. *P. monodon* may be the strongest osmoregulator among the *Penaeus* species so far studied.

Introduction

There are many species of *Penaeus* of which osmoregulatory abilities have been studied. These are *P. aztecus*, *P. carinatus*, *P. duorarum*, *P. esculentus*, *P. indicus*, *P. japonicus*, *P. merguiensis*, *P. plebejus*, *P. setiferus*, *P. stylirostris* and *P. vannamei* (Panikkar 1951; Williams 1960; McFarland and Lee 1963; Bursery and Lane 1971; Chu and Hanaoka 1975; Bishop et al. 1980; Dall 1981; Castille and Lawrence 1981a, 1981b, 1981c). In general, penaeid shrimp exhibit hyperosmotic regulation at low salinities and hypoosmotic regulation at high salinities with the isosmotic concentrations at 20-30 ppt. The juveniles are more euryhaline and stronger osmoregulators than the adults.

P. monodon and *P. penicillatus* were chosen for the present study due to their importance in shrimp culture in Taiwan (Liao and Chao 1983). The purposes of this study were: (1) to compare the osmoregulatory abilities of juvenile and adult *P. monodon* and *P. penicillatus* and (2) to compare the regulatory abilities of major ions in the hemolymph of these two species.

Materials and Methods

Shrimps of the two species used in this study were obtained from Tungkang Marine Laboratory. Sizes in carapace length were: *P. monodon* adults, 46.6 ± 2.4 mm; juveniles, 20.0 ± 1.6 mm; *P. penicillatus* adults, 26.9 ± 1.2 mm; juveniles 16.4 ± 0.9 mm.

Experimental salinities below 30 ppt were prepared by dilution of seawater with tap water. Higher salinities were prepared by adding concentrated seawater obtained by evaporation at 50°C. The salinity range and acclimation method were all similar to those described by Dall (1981). Experimental temperatures ranged from 24 to 28°C for all animals.

Blood samples were withdrawn by inserting disposable 1-ml plastic syringes and 26G needles into the pericardial cavity. The blood was delivered into a clean needle cap. Only intermolt animals were sampled.

Osmotic and ionic concentrations were determined in both hemolymph and seawater. Osmotic concentrations were measured with a Wescor vapor pressure osmometer, model 5130B, utilizing a 5-μl sample on a 6.35-mm diameter disc. Chloride samples were titrated with a Hiranuma chloride counter, model CL-53(M), utilizing a 20-μl sample in a 6-ml electrolyte. Sodium, potassium, calcium and magnesium concentrations were analyzed with a Hitachi atomic absorption spectrophotometer, model 170-30. Gas mixture was air-acetylene, used for these four ions with burner rotated across the light path to attenuate the strong absorption of sodium and magnesium. For these four ions a sample of 50μl was diluted to 35 ml with deionized water. A mixed standard (sodium, 435 mEq/l; potassium, 10 mEq/l; calcium, 30 mEq/l; magnesium, 20 mEq/l) and a standard seawater (chlorinity, 19.378 ppt) were used as a check. Juveniles were not used for ion measurement because of the difficulty in obtaining sufficient hemolymph for analysis.

Results

Results for juvenile *P. monodon* confirmed that it was a highly efficient osmoregulator. The curve was almost linear between 103 and 1,480 mOsm/kg (3-50 ppt). Adults were also efficient osmoregulators above 444 mOsm/kg (about 15 ppt). The isosmotic point was 730 mOsm/kg for juveniles and 750 mOsm/kg for adults. Juveniles of *P. penicillatus* were efficient osmoregulators between 157 and 875 mOsm/kg (5-30 ppt), with the isosmotic point at 747 mOsm/kg. Adults were efficient between 444 and 875 mOsm/kg (15-30 ppt), with the isomotic point at 800 mOsm/kg (Fig. 1).

The curves for sodium and chloride (Fig. 2) showed similar shapes to those for the osmotic concentrations of adults (Fig. 1). *P. penicillatus* was a poorer hyperionic regulator for both sodium and chloride than *P. monodon*, but the two species did not differ in hypoionic regulatory ability. The isoionic points for both sodium and chloride were a little higher in *P. monodon* (Na, 352 mEq/l; Cl, 320 mEq/l) than in *P. penicillatus* (Na, 348 mEq/l; Cl, 304 mEq/l).

Table 1 gives the percentages of the hemolymph osmotic pressures derived from sodium and chloride at each salinity. Together they accounted for 74-85% of the hemolymph osmotic pressures showing that sodium and chloride are the principal osmotically active solutes in the hemolymph. These precentages are higher in *P. monodon* than in *P. penicillatus* and are unaffected by the external salinity in both species. Although the concentration of chloride exceeds that of sodium in seawater, chloride in the hemolymph is not higher than that of sodium for *P. monodon* and is a little lower than that for *P. penicillatus*.

Both species regulated potassium efficiently over the tested salinity range and maintained a fairly constant level of potassium between 5 and 30 ppt. The curves turned upward roughly parallel to the isosmotic line above 30 ppt. The potassium concentrations in the hemolymph of *P. monodon* were a little higher than those of *P. penicillatus* over the tested salinity range. The isoionic points were 7.09 mEq/l for *P. monodon* and 6.08 mEq/l for *P. penicillatus* (Fig. 3).

Both species regulated calcium above external media, increasing progressively and coming near to isoionic line at 50 ppt. The calcium concentrations in the hemolymph did not differ between the two species. Both species strongly reduced magnesium concentrations below those of the external media over the tested salinity range, with no significant difference between the two species (Fig. 3).

Discussion

The results presented in this paper are similar to earlier studies of other *Penaeus* species (Castille and Lawrence 1981a, 1981b; Dall 1981; Dall and Smith 1981). However, *P. monodon* shows patterns of osmotic and ionic regulation most similar to those of *Metapenaeus bennettae* which is thought to be one of the most efficient osmotic and ionic regulators among the Natantia (Dall and Smith 1981). It indicates that *P. monodon* may be one of the most efficient osmotic and ionic regulators among the *Penaeus* species. This corresponds to the fact that the postlarvae of *P. monodon* can withstand salinity ranges of 0-60 ppt (Motoh 1981).

The osmoregulatory abilities of penaeid shrimp can be correlated with their salinity distribution (McFarland and Lee 1963). Motoh (1981) stated that the postlarvae of *P. monodon* settle on areas of almost 4 ppt in salinity as their nursery ground, supporting the statement that the more efficient osmoregulators distribute themselves abundantly in lower salinity (Castille and Lawrence 1981a). However, there are many factors other than salinity or osmoregulatory ability *per se* that might determine the salinity distribution of the shrimp (Dall 1981; Motoh 1981). Dall (1981) even concluded that osmoregulatory ability may not play a direct role in nursery ground selection by the juvenile penaeid shrimp, and suggested that *Penaeus* spp. postlarvae are all genetically highly euryhaline but may lose this ability with development in more stable environments. To prove this, it is necessary to compare the osmoregulatory abilities of penaeid shrimp reared from postlarvae in a range of salinities (Dall 1981).

Both *P. monodon* and *P. penicillatus* reduce their hyperosmoregulatory ability in their adult stage. Similar results are found in many other *Penaeus* spp. (Castille and Lawrence 1981b; Dall 1981). It is a common feature of euryhaline adult penaeid shrimp to migrate to offshore waters for spawning (Panikkar 1968; Motoh 1981). Castille and Lawrence (1981b) pointed out that this migration behavior is not directly necessitated by osmotic regulation since the adult animals are still capable of hyperosmotic regulation at salinities below the isosmotic point. However, the adults' migration to high salinity waters may be necesary for ovarian development (Chu and Hanaoka 1975; Oshiro 1984) and for embryonic and larval development (Lim 1982).

It has been suggested that the maximum growth of an organism occurs in isosmotic media, since the animal would be expending the least energy in doing osmotic work (Panikkar 1968). This may be true in *P. vannamei* and *P. stylirostris* (Huang 1983) but not in *P. aztecus*, *P. duorarum* and *P. setiferus* (Zein-Eldin 1963). However, the salinity *per se* has little effect on metabolic rate of euryhaline penaeid shrimp (Kutty et al. 1971; Liao and Huang 1975; Bishop et al. 1980; Gaudy and Sloane 1981), indicating that the energy required for osmoregulation may be relatively little. In *P. monodon*, the optimum salinity range for growth may change with their life history, with 10 ppt for early postlarvae (Valencia 1976), 15-20 ppt for late postlarvae (Chen 1984) and 15-25 ppt for juvenile (Chen 1984). In *P. penicillatus*, the maximum growth occurs in salinity of 15 ppt for late postlarvae (Chen 1984). These are all below the isosmotic points of late juveniles of these two species; however, their osmoregulatory abilities are most efficient over these salinity ranges (Fig. 1).

Castille and Lawrence (1981a) pointed out the percentages of hemolymph osmotic pressure derived from sodium and chloride were unaffected by external salinity in the species of subgenus *Litopenaeus*, but increased with external salinity in the species of subgenus *Melicertus*. In this study, these percentages were unaffected by external salinity in both species.

In *P. monodon* and *P. penicillatus*, the ratios of Na:Cl in hemolymph are higher than those in seawater. A similar result was obtained in five other *Penaeus* spp. by Castille and Lawrence (1981a). This could be due to the urine of *Penaeus* spp. which is isoionic to the hemolymph with respect to chloride, but hypoionic to the hemolymph with respect to sodium (Castille and Lawrence 1981c). In addition, the lower urinary sodium concentration may be due to replacement of sodium by magnesium (Castille and Lawrence 1981c).

In this study, potassium was strongly regulated at low salinities in both *P. monodon* and *P. pencillatus*. A similar pattern was found in other *Penaeus* spp. but their potassium levels tended toward accumulation (Dall and Smith 1981). Results of calcium and magnesium regulation are also similar to those of other *Penaeus* spp. studied, with the calcium levels of hemolymph higher, and the magnesium levels lower than those of the external medium (Dall and Smith 1981).

Acknowledgements

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Table 1. Percentage of the hemolymph osmotic pressure derived from sodium and chloride.

Salinity (ppt)	*P. monodon* Sodium	*P. monodon* Chloride	*P. monodon* Total	*P. penicillatus* Sodium	*P. penicillatus* Chloride	*P. penicillatus* Total
5	43	40	83	40	37	77
10	43	42	85	40	38	78
15	42	42	84	38	35	74
20	42	40	82	38	37	75
25	43	42	85	40	38	78
30	43	41	84	41	40	81
35	42	42	84	40	38	78
40	41	41	82	41	39	80
45	42	41	83	39	36	75
50	41	41	82	40	37	77

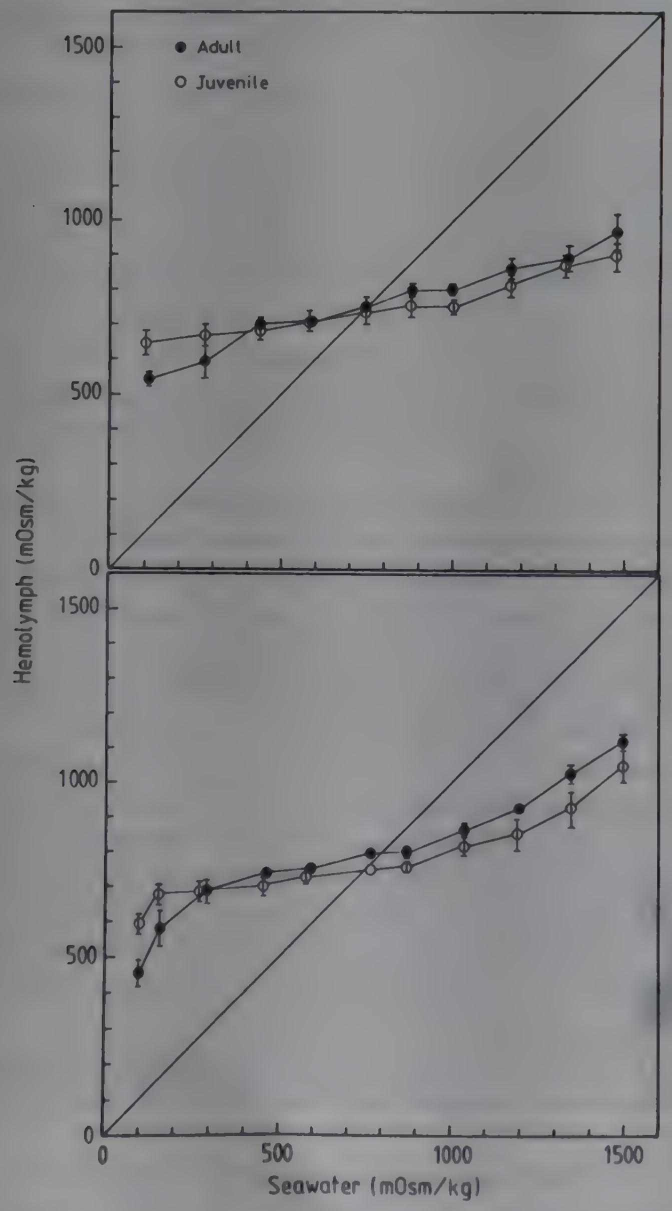

Fig. 1. Osmotic concentration of the hemolymph of *P. monodon* (top) and *P. penicillatus* (bottom) acclimated to different salinities.

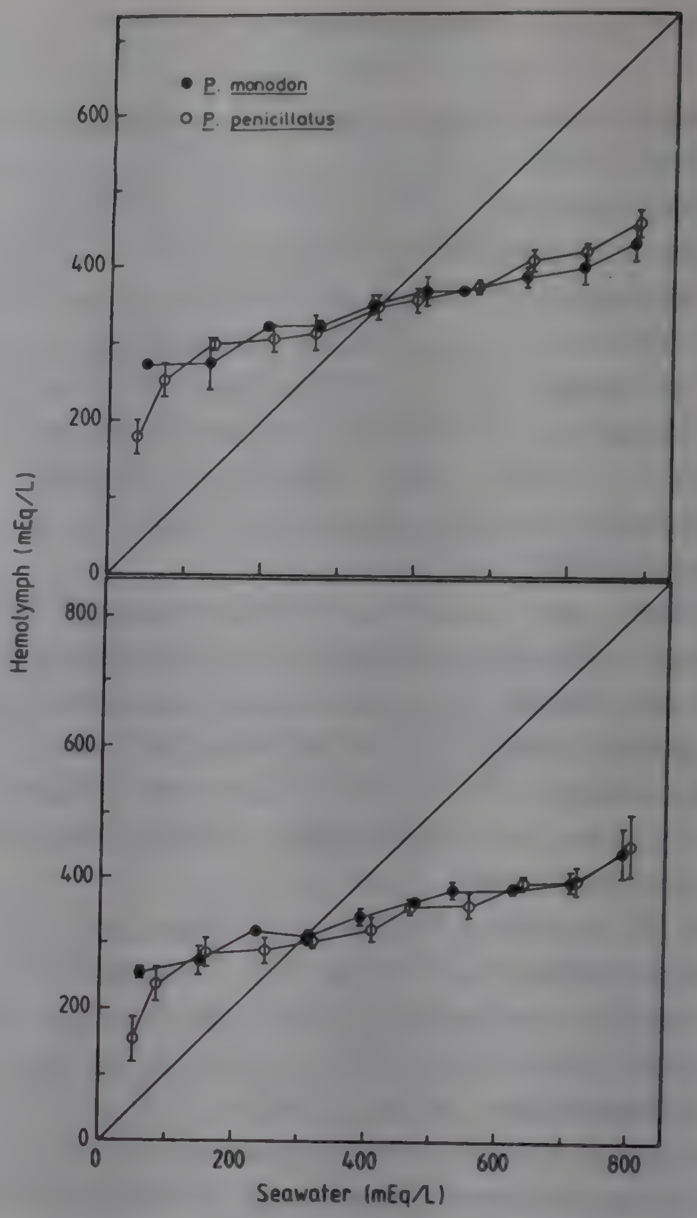

Fig. 2. Sodium (top) and chloride (bottom) concentrations of the hemolymph of *P. monodon* and *P. penicillatus* acclimated to different salinities.

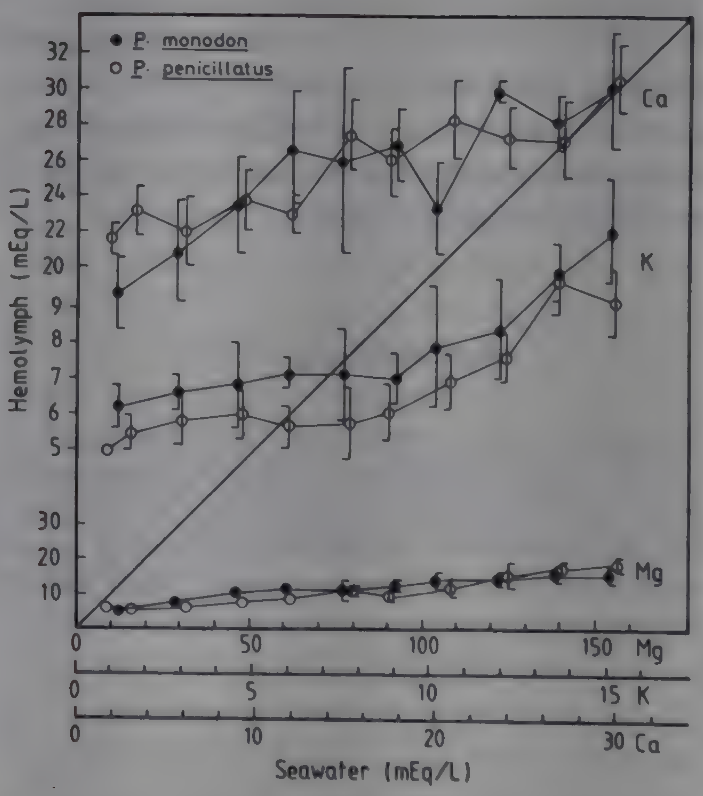

Fig. 3. Potassium, calcium and magnesium concentrations of the hemolymph of *P. monodon* and *P. penicillatus* acclimated to different salinities.

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Osmoregulation in *Penaeus monodon:* Effects of Molting and External Salinity

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Abstract

The effect of molting on osmotic, chloride, calcium and total protein concentrations in the hemolymph of the shrimp *Penaeus monodon* was investigated. Shrimp were stocked in various salinities and then sampled during molt and after molting. Regardless of medium salinity, tissue water as well as osmotic and chloride concentrations in the hemolymph became stable within one day after molting. In general, total protein concentrations remained stable throughout the molting cycle. Large fluctuations in hemolymph calcium were observed 0-6 hours after molt. In low salinities, hemolymph calcium peaked at 3 hours postmolt to values 30% higher than those during molt. These values subsequently decreased rapidly one day after molting, when hemolymph concentrations achieved intermolt values. At 44 ppt, calcium concentrations were highest during molt, then gradually declined by about 15% to intermolt values. At this salinity, shrimp were also dehydrated during molt, but % tissue water increased to normal intermolt values within one day after molting. The converse situation was observed in shrimp at low salinities.

Shrimp tended to conform more to the environmental osmolality 0-6 hours after molt than shrimp 12 hours to 14 days after molt. Isosmotic points were higher during molt than during intermolt. Hemolymph chloride was very well regulated below 20 ppt medium chloride regardless of molting stage. Above 20 ppt, newly molted animals tended to conform to the external chloride concentration when compared to intermolt stages. The isoionic points were independent of molt stage.

Tissue water and osmotic, chloride, and total protein concentrations in *P. monodon* varied with both molt stage and medium salinity. Dependence on external factors, however, gradually declined in older molt stages suggesting a reduction in integument permeability as the exoskeleton hardened. On the other hand, hemolymph calcium exhibited strong dependence on molt stage but not on medium salinity, suggesting that fluctuations in hemolymph calcium are largely influenced by internal requirements during ecdysis rather than integument permeability.

Introduction

Understanding the physiological response of shrimp to stress during molting and fluctuations in water salinity can improve survival and suggest better aquaculture methods. The physiological response by shrimp can be monitored by observing the changes in osmolality (number of particles such as ions, amino acids in solution) and ion concentration of the blood (hemolymph) relative to those of the medium. In dilute media (< 20 ppt), penaeids in intermolt keep their hemolymph more concentrated (more dissolved particles) than the environment; in high salinities (> 24 ppt), they tend to keep their hemolymph less concentrated than the medium (Castille and Lawrence 1981; Dall 1981; Dall and Smith 1981; Ferraris et al. 1986). Changes in hemolymph parameters, however, also occur because of endogenous factors, mainly molt stage (Robertson 1960; Bliss et al. 1966; Glynn 1968; Bursey and Lane 1971; Greenaway 1974). In spite of the strong possibility and relative importance of interactions between salinity and molting effects, these two factors have previously been treated in isolation, and we are not aware of any published investigation on the combined effects of salinity and molt stage on osmotic and ionic regulation in crustaceans. Studies on salinity effects generally avoided possible interactions with molting effects by using intermolt animals only.

In the wild, many crustaceans inhabit estuarine areas and during ecdysis are doubly stressed when salinities also change. In pond-cultured penaeids, the high mortalities, when molting coincided with large fluctuations in salinity, suggest that molting interacts to a large degree with salinity in inducing stress. By observing the response of several molt stages to different salinities, a better understanding of these interactions was expected.

Materials and Methods

A more detailed account of the methods used is found in Ferraris et al. (1986).

To minimize variation among experiments, only subadult shrimp (12-23 g) coming from one hatchery (SEAFDEC) and grown in nursery and grow-out ponds in the same locality (Leganes, Iloilo, Philippines) were used. Shrimp were transported to the laboratory and maintained in two 12-m^3 ferrocement tanks with aerated, filtered, flow-through 32 ppt seawater.

The interaction of molting and salinity effects on the hemolymph was observed in four salinities (8, 20, 32 and 44 ppt). These salinities were chosen based on results from previous experiments which showed that per cent survival was not significantly different among these salinities for this size of shrimp. One experimental series consisted of acclimatizing a batch of shrimp to one test salinity, then allowing them to molt in the same salinity. Shrimp were sampled as a function of time after molt and not molt stage. Using time after molt as an independent variable allowed the application of mathematical models to the data and making predictive statements. In subsequent statements, time after molt and molt stage were used interchangeably.

Because a significantly higher incidence of molt occurred one to two days after shrimp were transferred from maintenance to experimental tanks, the first molt in the experimental compartments was considered stress-induced, and all shrimp were allowed to complete at least one full molting cycle before they were sampled. On their second molt, the time of molt was noted. Since molting did not occur at the same time, each newly-molted shrimp was assigned a specific "sacrifice time" (corresponding to time after molt) at vandom. Prawns were sacrificed during, then 0.125, 0.25, 0.5, 1, 2, 4, 6, 10 and 14 days after molting. Throughout the entire series of experiments, water temperature ($28 \pm 0.5^{\circ}C$) and per cent daily mortality (2.0 ± 0.8) were constant.

Hemolymph from four animals was frozen, then pooled to constitute one replicate because a single animal yielded only 0.05-0.30 ml. Three replicates were collected per molting period in one salinity. Osmolality and chloride concentrations of the hemolymph or medium were determined by vapor pressure osmometry (Wescor 5100B) and mercurimetric titration (SIGMA) methods, respectively.

Initial values at molting, asymptotic values at intermolt and the time to reach asymptotic values from initial values at molt were analyzed by the modified Gauss-Newton method for nonlinear least squares using a hyperbolic model:

$$H_t = [P_1 (t) / (P_2 + t)] + P_3, \qquad (1)$$

where H_t = hemolymph osmolality or chloride concentration at any time t; P_1 = the difference between initial (during molt) hemolymph osmolality or chloride concentration values; and asymptotic (during intermolt) values so that when values during molt are less than those during intermolt, $P_1 > 0$; when values during molt are greater than those during intermolt, $P_1 < 0$; P_2 = time required to reach 50% (P_1); P_3 = initial values during molt.

From equation 1, when t = 0, then $H_0 = P_3$. The intermolt value is assumed to be asymptotic, and $(P_1 + P_3)$ = asymptote. The time required to reach a fraction y ($0 < y < 1$) of P_1 is computed by:

$$t_y = y (P_2)/(1-y) \qquad (2)$$

The parameter P_2 is a rate constant used to estimate how much time is necessary for hemolymph of newly molted shrimp in a certain salinity to approach intermolt values. P_2's, P_3's and $(P_1 + P_3)$'s were then compared using the Student's t-test.

The response of hemolymph osmolality and chloride of *P. monodon* of various molt stages to changes in salinity was determined by the slopes of the linear regression of hemolymph vs. medium osmolality and of hemolymph vs. chloride concentration. The slope is a measure of the prawns' ability to regulate internal hemolymph values independent of external fluctuations in salinity. Weak osmoregulators display a large slope; correspondingly, strong osmoregulators have a small regression coefficient. Isosmotic or isoionic points (where hemolymph = medium concentration) were calculated according to Ferraris et al. (1986). Isosmotic or isoionic points were then compared using the Student's t-test.

Results and Discussion

The response of *P. monodon* to a change in molt stage depended on salinity (Fig. 1). Thus, hemolymph osmolality increased during molting in high salinities, but decreased during molting in low salinities. Hemolymph osmolality in 44 ppt during intermolt (820 mOsm/kg; Fig. 1) increased by 44% ($P < 0.001$) during molt (1,170 mOsm/kg) until it approached that of ambient (1,220 mOsm/kg). At 32 ppt, the intermolt value of 690 mOsm/kg increased by 30% ($P < 0.001$) to about 900 mOsm/kg at molt, a value similar to that of ambient (890 mOsm/kg). Thus, in high salinities, the magnitude of increase in hemolymph osmolality at molt apparently increased as a function of external osmolality. Moreover, intermolt stages maintained hemolymph osmolality below that of the environment whereas in dilute media, intermolt *P. monodon* maintained intermolt osmolality above that of the medium. The change in hemolymph osmolality during ecdysis from that during intermolt in 20 ppt was small but still significant ($P < 0.025$). Hemolymph osmolality decreases significantly ($P < 0.025$) by 22% during molt at 8 ppt (520 mOsm/kg from 630 mOsm/kg during intermolt), but still remained significantly higher ($P < 0.001$) than the medium (220 mOsm/kg). In *Crangon crangon*, a strong hyper- and hyporegulator, hemolymph osmolality is also significantly reduced when ecdysis occurs in a dilute (10 ppt) medium (Hagerman and Larsen 1977). Hemolymph osmolality displayed a wider range of values during molt (520-1,170 m0sm/kg) than during intermolt (620-820 m0sm/kg). Results suggest that *P. monodon* tends to become isosmotic during or immediately preceding ecdysis because hemolymph osmolality tends to approach that of the medium (Fig. 1).

One of the limiting factors in the low salinity distribution and culture of *P. monodon* may be its ability to maintain a hemolymph osmolality ≥ 500 during molt, and ≥ 600 m0sm at intermolt (Fig. 1). On the other hand, the high salinity distribution and culture of this shrimp are constrained by its ability to reduce the hemolymph osmolality from values at molt to that in intermolt.

The response of *P. monodon* to a change in salinity is afected by molt stage. Newly-molted animals are very weak osmoregulators (large slopes), and changes in hemolymph osmolality closely parallel changes in external salinity. The relative shift from a weak to a strong osmoregulator is apparent when regression lines describing hemolymph vs. medium osmolality taken 0, 0.125 and 0.25 days after molt are compared to those taken > 0.5 days after. Newly-molted prawns display slopes twice as steep ($p < 0.05$) as those of intermolt stages.

In rock lobster, Dall and Smith (1978) suggested that the increased inward and outward water transport during molting is due simply to increased permeability of the soft integument. Permeability of the *P. monodon* integument is probably also very high during molt, and can account for the largely salinity-dependent fluctuations in hemolymph parameters as well as the apparent inability of the animal to regulate the hemolymph at this time. Because regulation of hemolymph osmolality is largely achieved within one day after molt, integument permeability at this time is presumably reduced as well. A reduction in permeability increases efficiency (less backflux) of ion excretion in high, and ion absorption in low salinities.

The significance of higher isosmotic points during molt as opposed to intermolt is not clear and may be indicative of a higher permeability to or a requirement for a higher concentration of ions during molt. Panikkar (1968) has hypothesized that crustaceans experience least osmotic stress and undergo best growth when placed in waters of isosmotic salinity. This suggestion assumes that isosmotic points do not change as a function of molt stage. Minimizing osmotic stress would require changing the medium osmolality according to equation 1 by letting ecdysis take place in 34 ppt (isosmotic point during molt), then gradually decreasing medium salinity to 24 ppt (isosmotic point during intermolt).

As in hemolymph osmolality, chloride at intermolt is regulated within a narrower range (310-450 mM), than that during molt (250-520 mM), and to the external chloride concentration (120-730 mM). Hemolymph chloride in newly-molted *P. monodon* is 15-30% higher ($P < 0.025$) than that during intermolt in both 32 and 44 ppt seawater (Fig. 2). When prawns are allowed to molt in isoionic salinities (20 ppt), there is no change in hemolymph chloride throughout the molting cycle ($P > 0.50$); at 8 ppt, hemolymph chloride decreases by 24% ($P < 0.001$) during molt. The direction of changes in chloride concentrations support our previous suggestion that *P. monodon* tends to become isosmotic during molt in high salinities, possibly by isosmotic fluid absorption. In low salinities, lowered chloride concentrations during molt again suggest osmotically-driven net water uptake which results in dilution of the hemolymph, a finding similar to that of Hagerman and Larsen (1977) for *Crangon vulgaris* allowed to molt in 10 ppt.

Hemolymph chloride is very well regulated below 20 ppt in all molt stages, and the isoionic point of hemolymph vs. medium chloride is the same regardless of molt stage ($P > 0.10$). The pooled isoionic point is 301 ± 6 mM, which corresponds to an external salinity of 19 ppt. There is a slight difference ($p < 0.05$) in slopes of hemolymph vs. medium (20-40 ppt) chloride concentrations between molt and intermolt stages. Thus, *P. monodon* at molt regulates chloride better than it does osmolality. Using equation 2, we can predict how much time is required for hemolymph osmolality or hemolymph chloride to change to intermolt values. The $t_{0.75}$ values (when hemolymph values are already asymptotic in many cases, and no longer significantly different from intermolt values) for 8, 20, 32 and 44 ppt were 1.4, 0.0, 1.0 and 1.1 days, respectively. These represent the amount of time it takes for shrimp to reduce the difference between hemolymph osmolality during molt and hemolymph osmolality at intermolt by 75% ($0.75 \times P_1$). For chloride, $t_{0.75}$ for shrimp in 8, 20, 32 and 44 ppt were 0.4, 0.0, 0.6 and 2.5 days, respectively.

Shrimp molting in extremely high (or low) salinities may require more time and energy in normalizing hemolymph osmolality because they would be secreting (or absorbing) ions against a higher ionic gradient. This long time interval increases the vulnerability of prawns to predation and cannibalism, and prolongs their inability to forage for food. In aquaculture conditions, it is advantageous to allow *P. monodon* to molt near isosmotic and isoionic conditions.

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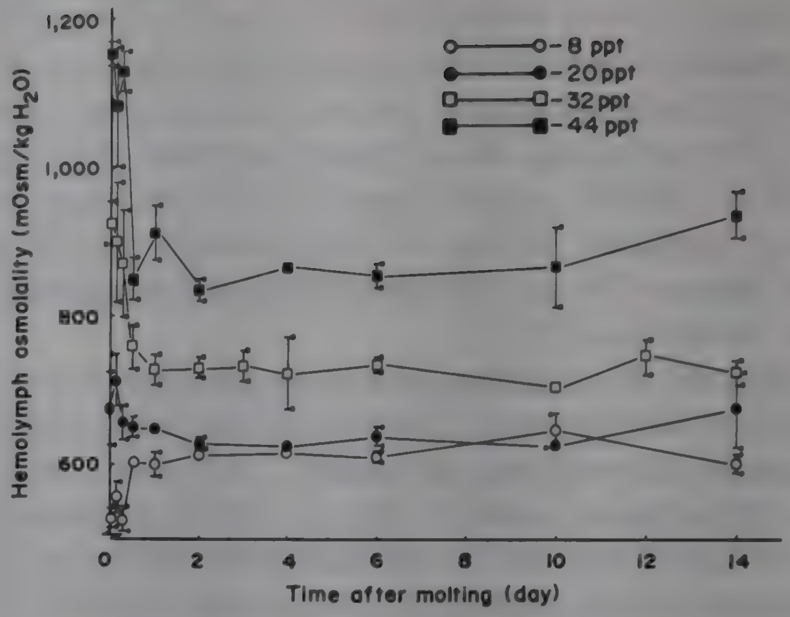

Fig. 1. Changes in hemolymph osmolality as a function of time after molting in 8, 20, 32 and 44 ppt. Each point represents the mean and the bars ± 1 standard error of the mean (SEM) of three determinations (4 shrimps per determination). SEM's less than the size of the symbols are not shown.

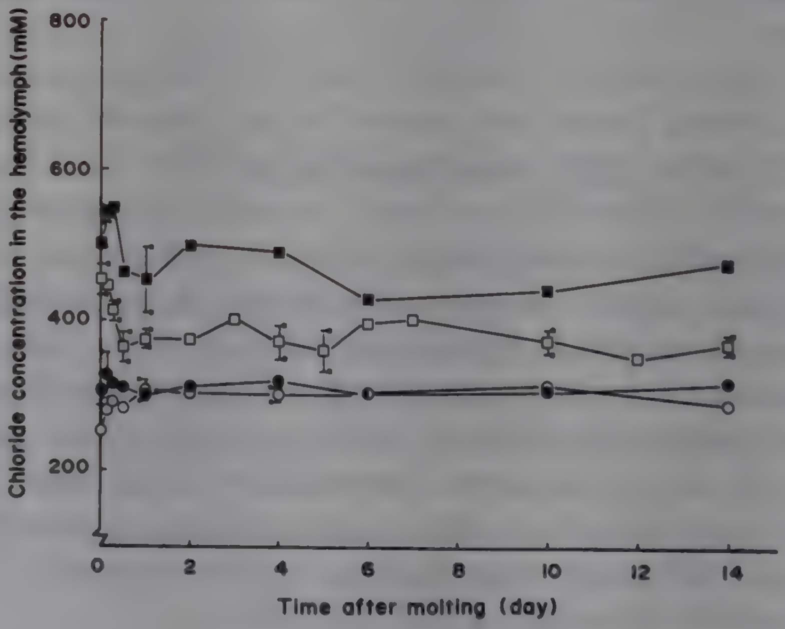

Fig. 2. Chloride concentration in the hemolymph as a function of time after molting in 8, 20, 32 and 44 ppt. Definition of symbols as in Fig. 1.

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Effects of Dissolved Oxygen, Temperature and Salinity on the Oxygen Consumption of the Grass Shrimp, *Penaeus monodon*[1]

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Abstract

The resting rate of oxygen consumption by individual *Penaeus monodon* over a size range of 0.4-80.0 g at three different temperatures (20, 25 and 30°C), six salinities (3, 5, 15, 25, 35 and 45 ppt) and a range of dissolved oxygen (DO) levels (less than saturation point) was measured in a closed system using a polarograph oxygen meter (YSI Model 57). The shrimp were starved for 24 hours and held in the respiration chamber for 3 hours prior to the commencement of each experiment. Oxygen consumption was found to be independent of DO over 4.0 mg/l at 30 ppt and 20, 25 and 30°C. Oxygen consumption was reduced by 7.4-11.9%, 22.2-35.3% and 63.0-71.4%, respectively, when DO was 3.0, 2.0 and 1.0 mg/l. The general allometric equation was used to describe the relationship between the rate of oxygen consumption (R) and body weight (W) when DO was greater than 4.0 mg/l. The equations were: $R = 0.168 \times W^{0.853}$ (20°C); $R = 0.311 \times W^{0.863}$ (25°C) and $R = 0.487 \times W^{0.881}$ (30°C). Salinity had no measurable effect on oxygen consumption within the tested range. However, at 3 ppt, shrimp less than 1.3 g showed greater tolerance to DO levels than shrimp larger than 9.2 g.

Introduction

Penaeus monodon is a tropical penaeid shrimp of considerable commercial importance. Establishment of larval rearing techniques (Liao et al. 1969) has enabled the monoculture of this species and its production from aquaculture has been increasing rapidly mainly in Southeast Asian countries (Kungvankij 1985; New and Rabanal 1985). This has stimulated many aquaculture-oriented studies of *P. monodon* in recent years.

Several studies dealing with oxygen consumption of *P. monodon* have been limited to either postlarvae (Liao and Huang 1975; Gaudy and Sloane 1981; Licop 1985) or juveniles and subadults between 1.5 g and 18.0 g (Ting 1970; Kuwabara et al. 1985). This paper aimed to study the respiration characteristics of juvenile to adult *P. monodon* in relation to dissolved oxygen (DO), temperature and salinity.

Materials and Methods

Because the shrimp were both cultured (0.4-42.0 g) and wild (43-80 g), a pretreatment of three to five weeks was necessary to eliminate possible variation due to their source. During this period, they were kept in outdoor tanks (30 t; 30-33 ppt) and were fed fresh oyster, squid and prawn. Shrimp were then transferred to indoor tanks (40-100 l) for acclimation at 20, 25 and 30°C. Salinity was adjusted by 5 ppt daily by dilution with distilled water or by adding hypersaline seawater. The shrimp were acclimated at the test salinities of 3, 5, 15, 25, 35 and 45 ppt and temperature combinations for a minimum of one week prior to any experiment. Test temperatures and salinities were maintained between ± 0.5°C and ± 1.5 ppt, respectively. Photoperiod was kept at 12L:12D, the photophase starting at 6:30 a.m. Shrimp were starved 24 hours before testing, but otherwise fed commercial formula pellets three times daily throughout the indoor phase.

Individual shrimp were placed in the unsealed respiration chamber (Fig. 1) 3 hours prior to the commencement of DO measurements. Preliminary experiments showed that 3 hours was adequate to avoid an overestimation of the basic metabolic rate due to handling effects (Fig. 2). The total volumes of the closed systems were 1.0, 2.6 and 5.33 l for shrimp of 0.3-10.0 g, 10.1-40.0 g and 40.1-80.0 g, respectively. The flow rate in the closed system was 10-15% of the volume per minute. The oxygen level was monitored at 10- to 60-min. intervals, depending on the rate of decline in oxygen, using a polarograph oxygen meter (YSI Model 57). The accumulation of ammonia-N in the closed system was monitored in the test runs and was shown to be below 1.3 ppm during the test phase. This concentration is well below toxic levels (Chen et al., this vol.). All measurements were taken during daytime under dim light condition (5-10 lux). Salinity effects on three size groups (0.6-1.3 g, 9.2-12.0 g and 25.6-34.3 g) were examined by running four replicates at

30°C. The results were subjected to two-way analysis of variance (Sokal and Rohlf 1981).

The oxygen consumption was calculated as follows:

$$C = (M_1 - M_2 - M_3) \times (V - P)/1{,}000 \times 60/t$$

where C is the oxygen consumption per hour (mg/hour), M_1 is oxygen concentration at time t_1 (mg/l), M_2 is oxygen concentration at time t_2 (mg/l), M_3 is the background oxygen consumption (mg/l) (i.e., the oxygen consumption in the system without the shrimp), V is the total volume (ml) of the closed system, P is the volume (ml) of the shrimp and t is the time (min.) between t_1 and t_2. Volume of a shrimp was estimated by multiplying the body weight by 0.93 (Fig. 3).

Results

The results indicated that the rate of respiration remains constant at DO level above 3.0-4.0 mg/l. Respiration then gradually decreases until the shrimp become moribund at DO level of 0.4-0.7 mg/l (Fig. 4).

Surfacing response (marked with arrows in Fig. 4) was first observed when DO reached 1.5-2.1 mg/l. Below this level, the frequency of intermittent surfacing increased as the oxygen depletion progressed. Duration of each surfacing was inconsistent, which probably contributed to the variation in the DO level at which shrimp became moribund. In this experiment, the complexity of the DO level and exposure time interaction prohibited the accurate estimate of the lower limit of oxygen tolerance.

The relationship between oxygen consumption and DO (Fig. 5) shows that the incipient limiting DO level is 4.0-4.3 mg/l, above which the resting rate of oxygen consumption was dependent on water temperature (Table 1), such that the weight-specific oxygen consumption was 1.14 mg/g/hour (20°C), 2.87 mg/g/hour (25°C) and 5.32 mg/g/hour (30°C). These figures were reduced by 7.4-11.9%, 22.2-35.3% and 63.0-71.4% when the DO concentrations were 3.0 mg/l, 2.0 mg/l and 1.0 mg/l, respectively.

The oxygen consumption per unit body weight (mg/g/hour) tended to decrease as the body weight increased (Fig. 6). Therefore, the results are best described by an allometric equation such that at the three temperatures (Fig. 7):

$$R = 0.168 \times W^{0.853}\ (20^{\circ}C) \quad \ldots 1)$$

$$R = 0.311 \times W^{0.863}\ (25^{\circ}C) \quad \ldots 2)$$

$$R = 0.487 \times W^{0.881}\ (30^{\circ}C) \quad \ldots 3)$$

where R is oxygen consumption by individual shrimp (mg/hour) and W is body weight (g). The thermal coefficient (Q_{10}) was also a function of body weight; $Q_{10} = 3.43 \times W^{0.02}$ (for temperature change from 20°C to 25°C) and $Q_{10} = 2.45 \times W^{0.036}$ (for temperature change from 25°C to 30°C). Estimates of Q_{10} values at various body weights indicate that temperature increments/decrements between 20 and 25°C have greater impact on shrimp than those between 25 and 30°C (Table 2).

Oxygen consumption was independent of salinity over the range of 5-45 ppt, but displayed a trend to decrease at 3 ppt (Fig. 8), which was significant at the 5% level (Table 3). Shrimp larger than 9.2 g, however, were less tolerant of low oxygen concentrations at 3 ppt exhibiting residual DO levels of 1.8 ± 0.4 mg/l compared to 0.6 ± 0.1 mg/l at higher salinities of 5-45 ppt (Figs. 9 and 10).

Discussion

In the present study, the oxygen consumption rate was constant until the incipient limiting oxygen tension was lowered to 4.0-4.3 mg/l. The same pattern was also observed by Dall (1986) and the present results showed that this threshold is unaffected by temperature. This means that progressively higher percentage saturation is required at higher temperatures to fulfill the oxygen requirements.

Studies by Liao and Huang (1975) and Chen (1985) reported that the oxygen consumption of postlarvae decreased when DO level fell below 3.8 and 4.0 mg/l. These results suggest that the oxygen regulatory system of *P. monodon* is well developed at early postlarval stages and that oxygen consumption is independent of DO at higher DO levels. The present results are also in accordance with other studies on salinity requirements (Bridges and Brand 1980). Ferraris et al. (1985) reported that young *P. monodon* show efficient osmoregulation over a salinity range of 5-55 ppt. In this study, the oxygen consumption was also stable over the tested range. This is in agreement with the known euryhalinity of this species (Cheng and Liao, this vol.). Temperature influences the respiration more than salinity.

Paloheimo and Dickie (1966), in their review, noted that the relation between oxygen consumption of animals (C) and their body weight (W) is, in general, very stable and can be expressed by a power function:

$$C = \alpha W^{\beta} \quad \ldots 4)$$

The two parameters, α and β, are, respectively, known as the level of metabolism and the weight exponent. Although Beamish (1964) reported that the value of β is species specific, Rao's (1958) data clearly show that it varies considerably depending on the experimental conditions. In this study, the value of β ranged from 0.853 to 0.881 which is close to the mean value of β (0.850) for decapod crustaceans (Weymouth et al. 1944). If the results in Fig. 7 are plotted on a log-log scale, they become three parallel lines ($P < 0.001$, ANOVA; Sokal and Rohlf 1981) and thus give the pooled value of

$\beta = 0.863$ ... 5)

compared to $\beta = 0.815$ for *Penaeus esculentus* (Dall 1986). The values of α in equations (1), (2) and (3) are directly related to water temperature, and a regression of α on temperature (t) gives a straight line:

$\alpha = -0.476 + 0.032 \times t$ ($r = 0.998$, $n = 3$) ... 6)

By incorporating equations (5) and (6) into equation (4), we obtain

$C = (-0.476 + 0.032 \times t) \times W^{0.863}$ ... 7)

which is best described by a three-dimensional figure (Fig. 11). Since the value of β varies with the experimental conditions (Rao 1958), more studies are required to determine the range of temperatures, salinities and body sizes within which this equation remains valid. Other variables such as sex may have a significant effect. It would also be of interest to determine whether or not this relationship can be applied to other penaeid species.

Further research also needs to be done on the active rates of oxygen consumption at low temperatures and the long-term effects of DO levels less than 4.0 mg/l as this information may help in improving shrimp culture techniques and may be beneficial to the development of live transportation methods of *P. monodon*.

Acknowledgements

The authors express their sincerest appreciation to those researchers and technicians at Tungkang Marine Laboratory who helped with these experiments. In particular, we are grateful to Adam Body and Dr. Derek Staples for their valuable comments on the manuscript. The research was supported by the Taiwan Provincial Government. [Grant No. 56.09006.1.03-08(6)].

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Table 1. Resting rate of oxygen consumption in relation to water temperature at 30 ppt.

Water temperature (°C)	20	25	30
Oxygen consumption (mg/g/hr)	1.14	2.87	5.32

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[1] Contribution A No. 65 from the Tungkang Marine Laboratory.

Table 2. Estimates of thermal coefficient (Q_{10}) at various body weight.

Temperature range	Body weight 5 g	10 g	20 g	40 g
20-25°C	3.54	3.59	3.64	3.69
25-30°C	2.60	2.66	2.73	2.80

Table 3. Two-way analysis of variances with four replicates. Size group (0.6-1.3 g, 9.2-12.0 g and 25.6-34.3 g) is treated as a fixed factor.

Source of variance	S.S.	d.f.	M.S.	F.	
Size group	0.199	2	0.10	30.06	$P < 0.01$
Salinity	0.041	5	0.001	2.50	$P < 0.05$
Interaction	0.030	10	0.003	1.00	N.S.
Error	0.179	54	0.003	–	
Total	0.450	71	–		

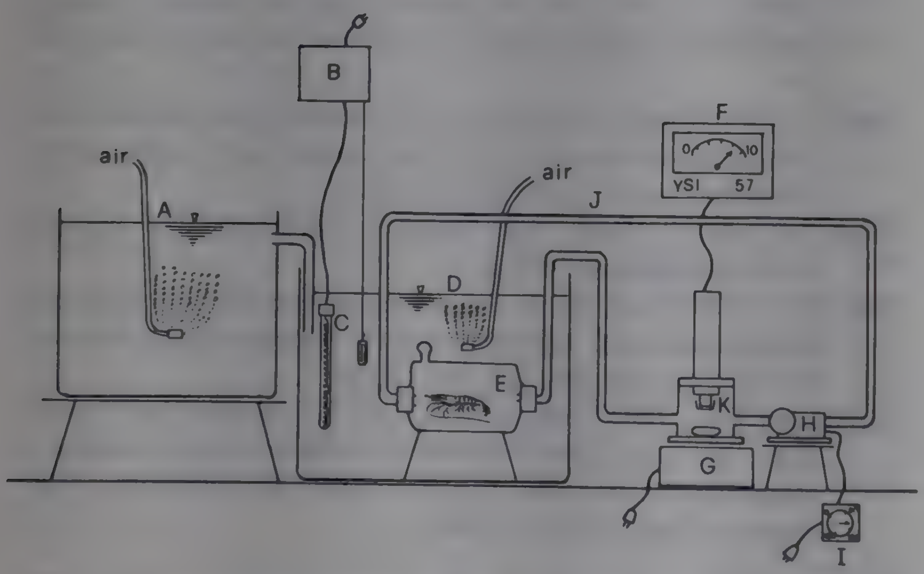

Fig. 1. Experimental apparatus used to measure the respiration of *Penaeus monodon.* The glass chamber (E) is sealed with rubber bungs to form a closed system, within which water circulation is maintained at the rate of 10-15% per minute. A: Holding tank (100 ℓ), B: Thermostat, C: Heater, D: Water bath (50 ℓ), E: Glass respiration chamber, F: Oxygen meter, G: Magnetic stirrer, H: Water pump, I: Transformer, J: Rubber tube, K: Acrylic stand.

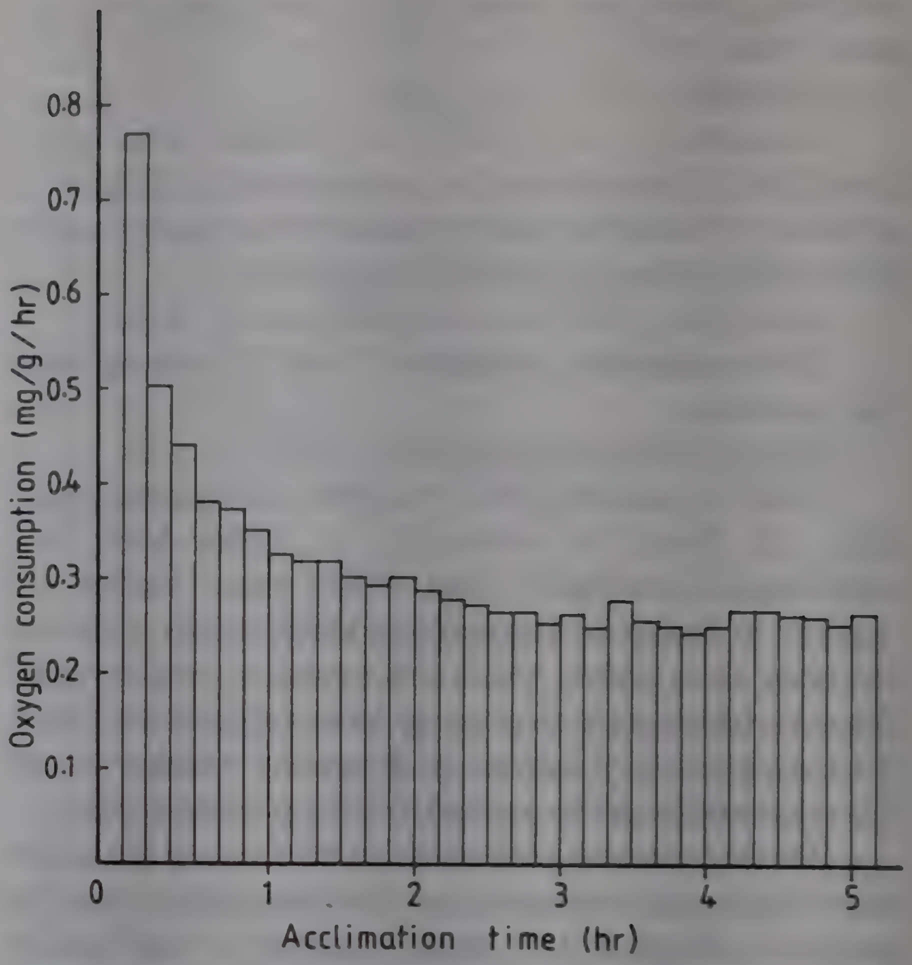

Fig. 2. Acclimation time required to minimize handling effect (prawn size 10.5 g; 20°C; 30 ppt).

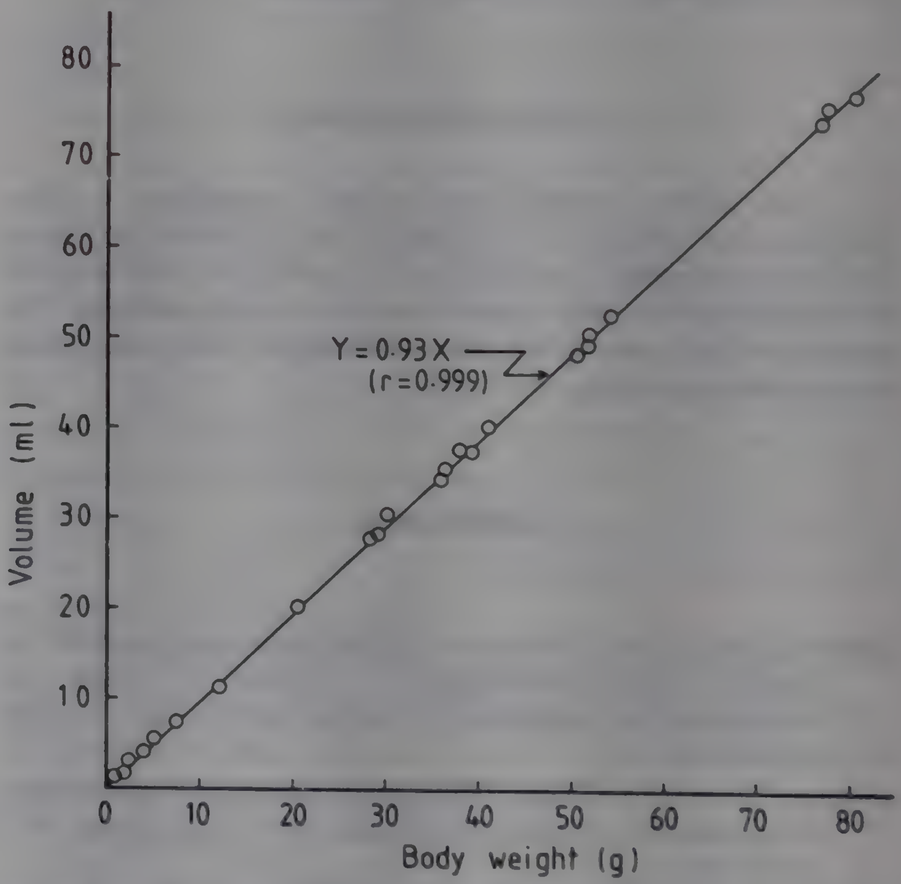

Fig. 3. Linear relationship between volume (ml) and body weight (g) of *Penaeus monodon* at 30 ppt.

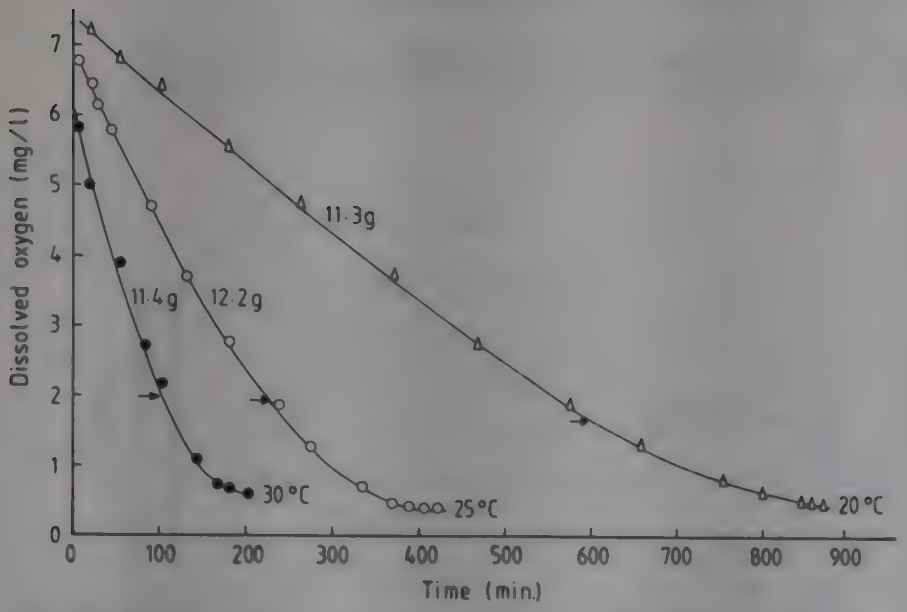

Fig. 4. Time course of oxygen depletion by *Penaeus monodon* in closed system (2.6 ℓ) at 25 ppt. The first surfacing response occurred at oxygen concentration of 1.7-2.0 mg/l (marked with arrows) and continued intermittently thereafter.

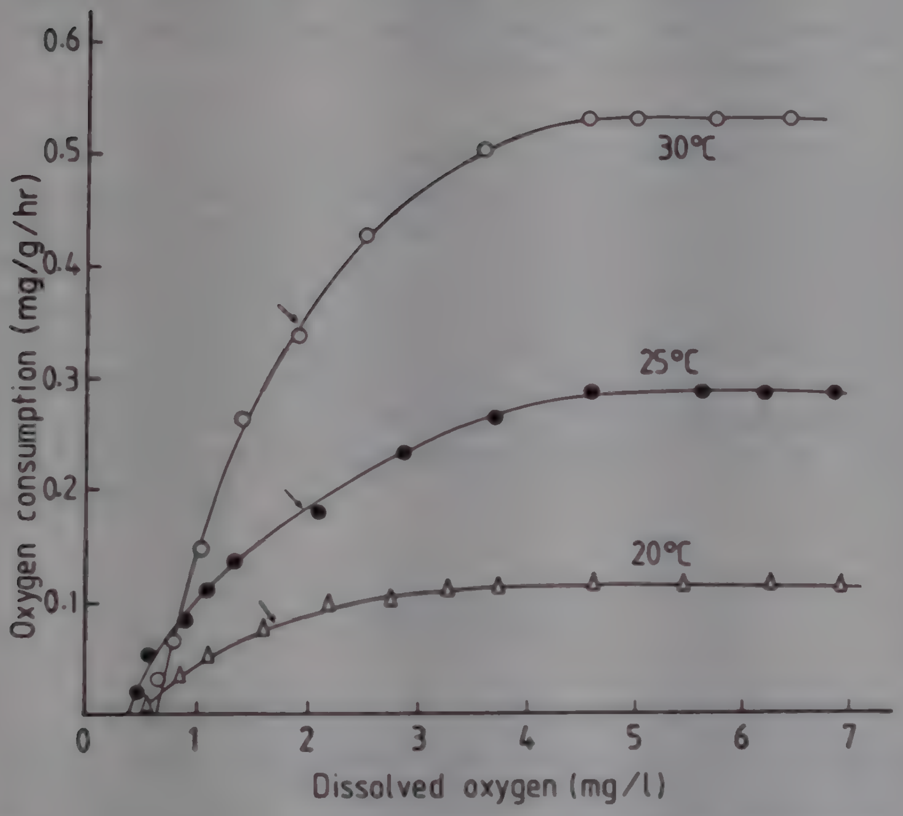

Fig. 5. Oxygen consumption (mg/g/hr) of *Penaeus monodon* at 25 ppt in relation to dissolved oxygen level at three temperatures. Arrows denote the D.O. level of the first surfacing response.

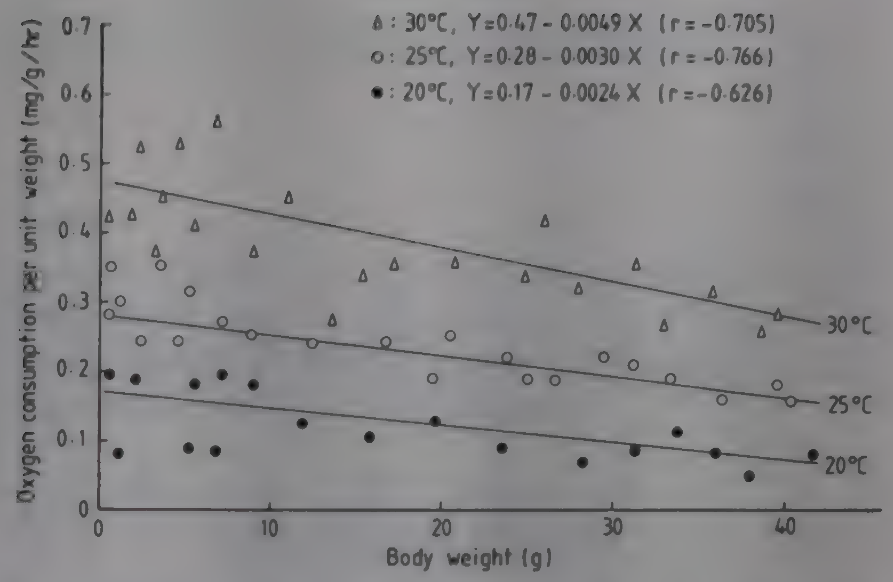

Fig. 6. Relation between oxygen consumption per unit weight (mg/g/hr) and body weight (g).

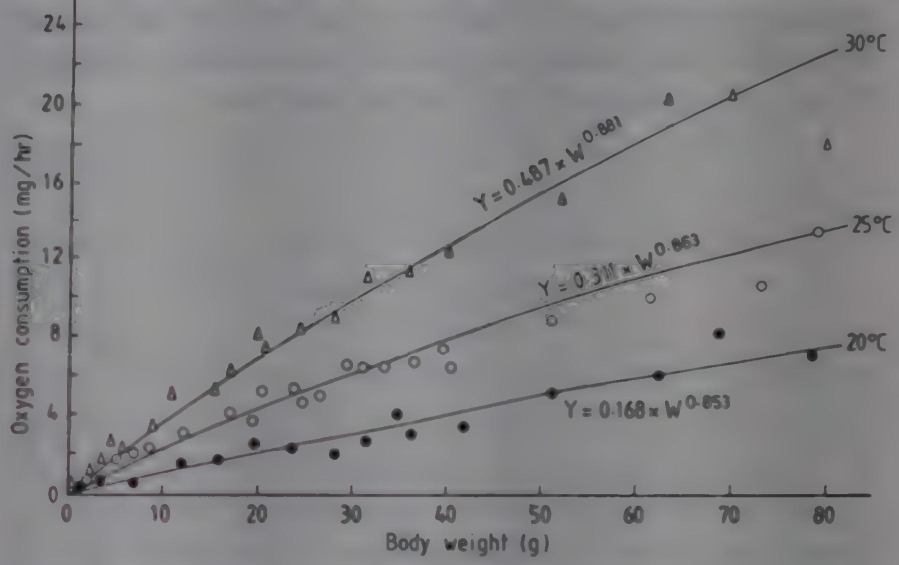

Fig. 7. Allometric relation between oxygen consumption and body weight of *Penaeus monodon* at three temperatures.

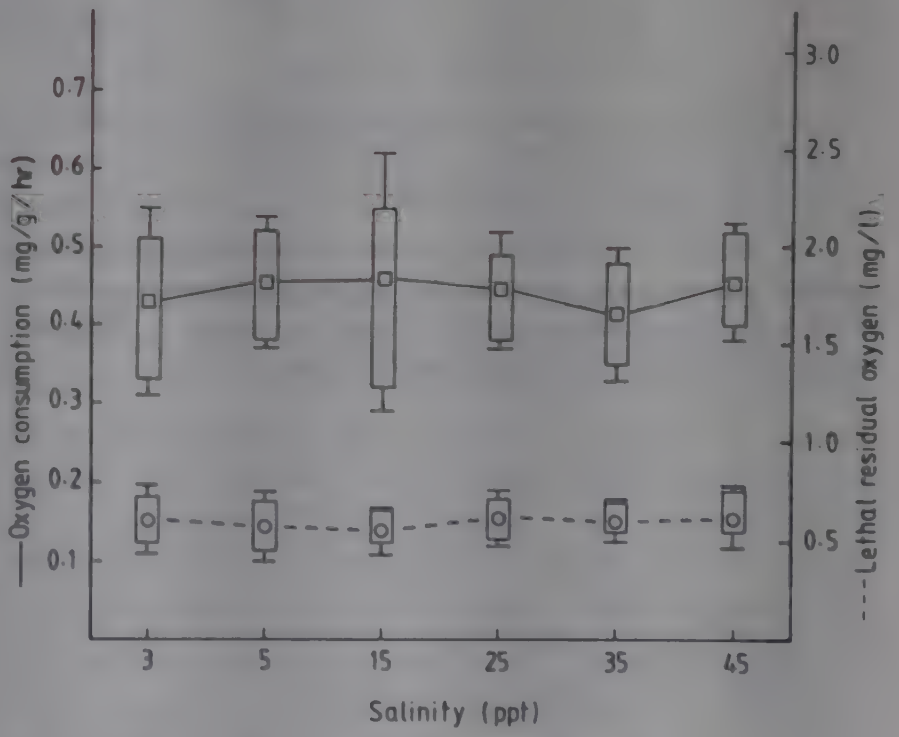

Fig. 8. Resting respiration rate (solid line) and oxygen level for hypoxia (dotted line) over salinity range of 3-45 ppt for juvenile shrimp (0.6-1.3 g): presented in the graph are mean ± 95% confidence limit (vertical bar) and observed range (box).

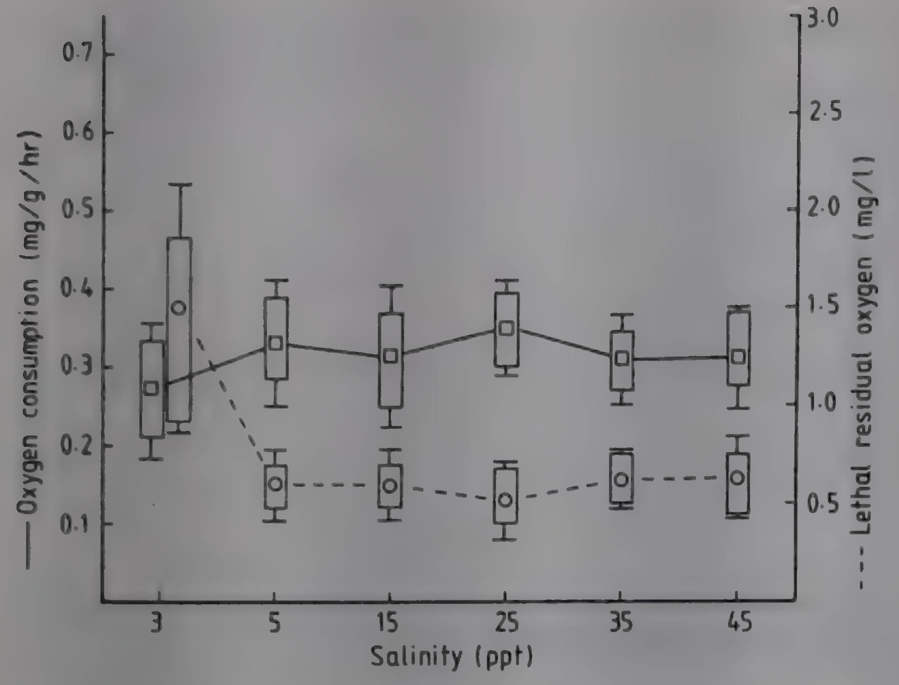

Fig. 9. Resting respiration rate (solid line) and oxygen level for hypoxia (dotted line) over salinity range of 3-45 ppt for sub-adults (9.2-12.0 g): presented in the graph are mean ± 95% confidence limit (vertical bar) and observed range (box).

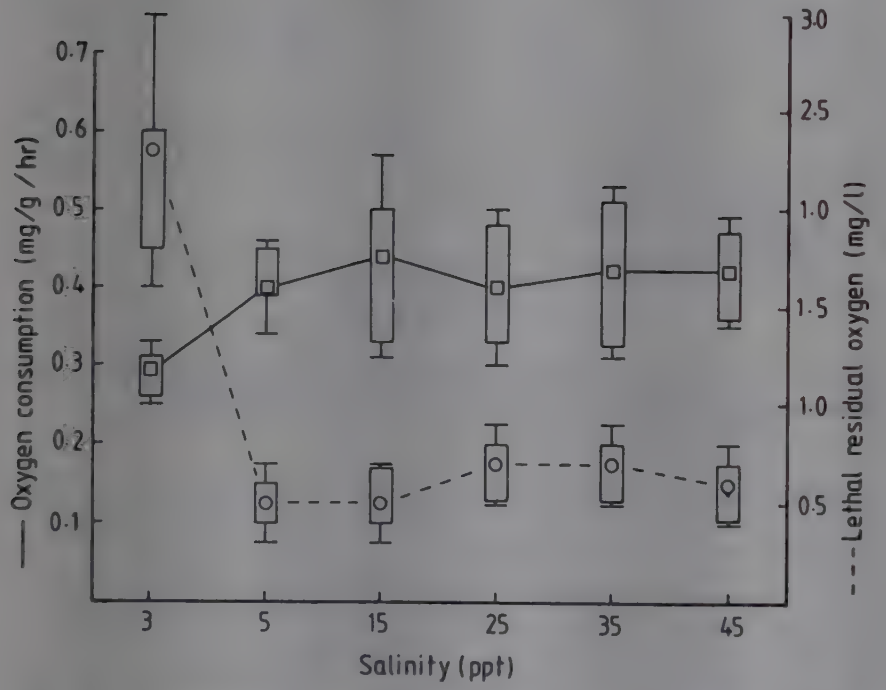

Fig. 10. Resting respiration rate (solid line) and oxygen level for hypoxia (dotted line) over salinity range of 3-45 ppt for prawns of 25.6-34.3 g: presented in the graph are mean ± 95% confidence limit (vertical bar) and observed range (box).

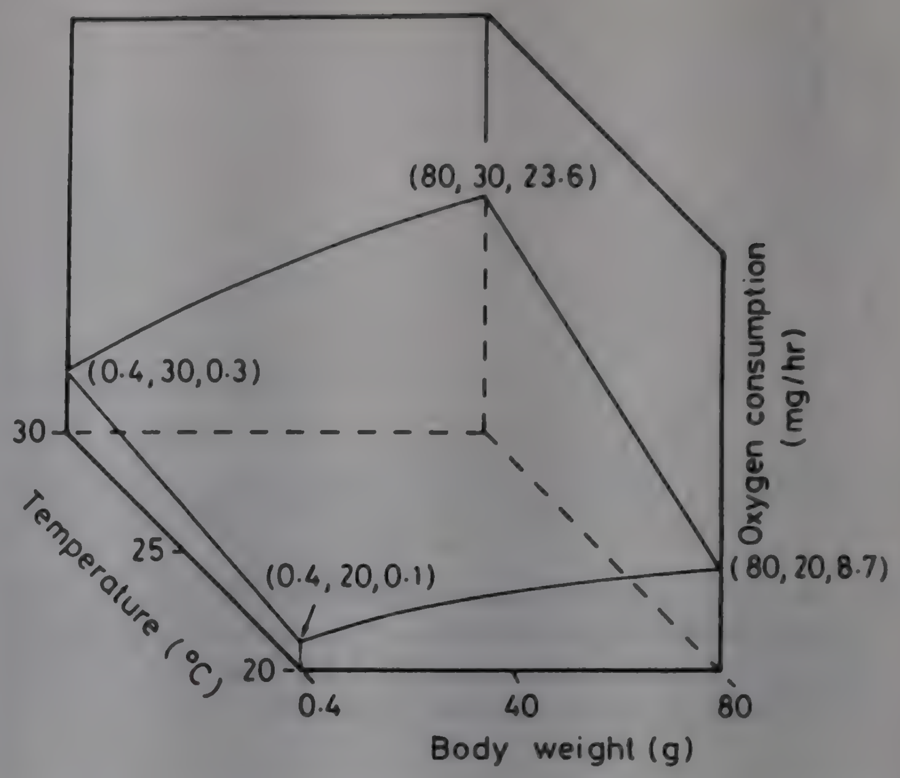
(80, 30, 23·6)
(0·4, 30, 0·3)
(0·4, 20, 0·1)
(80, 20, 8·7)
30
25
20
Temperature (°C)
0·4
40
80
Body weight (g)
Oxygen consumption (mg/hr)

Fig. 11. Three dimensional diagram for body weight — temperature — respiration relation: respiration (the Z axis) increases allometrically along the body weight (the X axis) and linearly along temperature (the Y axis).

Ontogenetic Changes in the Composition and Energy Budget of *Macrobrachium malcolmsonii*

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Abstract

In the course of embryonic development, *Macrobachium malcolmsonii* was found to exhibit progressive increases in water (56.7 to 75.6%), ash (2.6 to 11.7%) and carbohydrate (5.40 to 12.21%) and steady decreases in protein (50.11 to 42.61%), fat (41.9 to 33.5%) and potential energy (27.92 to 22.24 KJ/g dry wt). Fat served as the main source of energy for embryonic development. The cumulative yolk utilization efficiency during development was 85.6% on a dry weight basis. The metabolic rate of developing eggs was high during the initial and final stages of development whereas it was low during the mid-development stage.

Introduction

Developing larvae of crustaceans are known to absorb water throughout the period of development (Pandian 1970a, 1970b; Katre 1977). With increasing water content, the specific gravity of the larva decreases (Herring 1974), which allows the larva to reach the surface water.

Changes in the biochemical composition and potential energy during egg development have been studied in three species of *Macrobrachium*. Vijayaraghavan and Easterson (1974) made a study on energy utilization in developing eggs of the estuarine prawn *M. idella* and concluded that protein is the major source of energy during egg development. Katre (1977) reported that the freshwater prawn *M. lamarrei* obtains more than 90% of energy for embryonic development from fat oxidation. Working on *M. nobilii*, Balasundaram and Pandian (1982) restricted their studies to changes in the energy content of the developing eggs and did not report the changes in the chemical composition. The present paper reports the changes in the chemical composition and potential energy in the developing eggs of *M. malcolmsonii* (H. Milne Edwards).

Materials and Methods

Healthy individuals of *M. malcolmsonii* were collected from the river Cauvery (South India). Pairs were maintained in 40-l glass aquaria. Egg production ranged from 3,500 to 64,000 per female. At the acclimation temperature of 29 ± 1°C, the eggs required about 15 days to complete development. The following arbitrary developmental stages were chosen for chemical analysis:

Stage I (0 hour). Eggs soon after spawning, orange yellow in color; cleavage commences as furrows; eggs oval in shape and measure 520 x 432μm.

Stage II (120 hours). Eggs indicating differentiation of the blastoderm at one end; blastoderm occupies nearly 20% of the egg surface area; color of the eggs gradually becoming lighter; egg size 540 x 441μm.

Stage III (240 hours). Color gradually becoming light grey; pigmented eyes formed; cephalic appendages twitching occasionally; egg size 573 x 468μm.

Stage IV (larva). Freshly hatched zoea; total length from the tip of the rostrum to the tip of the telson 2.075 ± 0.210 mm.

At stage I, eggs from the females were removed immediately after spawning. At stages II and III, eggs were removed after the 5th or 10th day of spawning. Freshly-hatched zoeae were collected immediately after hatching for analysis.

One thousand to 5,000 eggs or larvae of each of the four stages were counted. Eggs were collected from females of 10 to 11.5 cm size only to avoid variation in size of the test eggs. After blotting the eggs or larvae, samples were separately weighed in a monopan balance (10 μg accuracy). Water content was determined by weighing the test material before and after drying at 90°C to weight constancy. Ash content was estimated by keeping the sample in a crucible of known weight in a muffle furnace of 560°C for a period of 5 hours as recommended by Paine (1964).

Protein was determined following the method of Lowry et al. (1951) and fat according to Raymont et al. (1964). Carbohydrate content was calculated from ash protein and fat contents. Calorific content was determined with a Parr 1421 microbomb calorimeter. Following the method of Pandian (1967) the "cumulative efficiency" was calculated: "body formed/body formed and yolk used for metabolism."

Results

Weight of a freshly spawned eggs of *M. malcolmsonii* averaged 60.90μg, of which 34.50μg was water. Water intake of 69.90μg increased the weight of the freshly-hatched zoea to 92.50μg (Table I).

The mean dry weight of an egg decreased from 26.40 μg in stage I to 23.50μg in stage III and, on hatching as a zoea, to 22.60μg. Hence the total loss during the whole embryonic development amounted to 3.80μg (Table 1). Although similar-sized females were chosen for egg collection, the size of the eggs varied up to 6% from the mean value.

Percentage composition of water, ash, protein, fat, carbohydrate and the energy content in different developmental stages of the egg are presented in Table 1. Proportions of ash, water and carbohydrate were significantly more in stage IV (zoea) than in stage I (egg). However, the reverse was true for the values obtained for protein, fat and energy. Data in Table 1 were statistically analyzed using one-way ANOVA test (Zar 1974). The analysis revealed that all the changes were statistically significant.

From the data, the changes in the chemical composition and energy content of a single egg from stage I to stage IV were calculated (Table 1). During the course of embryonic development, there was an increase in water, ash and carbohydrate and a decrease in protein and fat. The cumulative yolk utilization efficiency on a dry weight basis was 85.6%; the corresponding values were 77.6, 72.8, 68.4 and 68.2% for organic substance, protein, fat and energy, respectively.

Discussion

Most of the water uptake in *M. malcolmsonii* occurred in the zoae stage (Table 1). In *M. lamarrei* (Katre 1977), water intake is more constant during development, from 145μg in stage II to 180μg in stage II and almost the same in stage IV.

M. malcolmsonii utilized fat as the main source of energy from embryonic development. Of the total energy (0.2341 J) spent during embryonic development by an individual egg, 58.5% was obtained from fat oxidation while 36.2% was from protein oxidation. This observation supports the suggestions of Fluchter and Pandian (1968) and Pandian (1970a, 1970b, 1972) that, in general, demersal eggs utilize fat as major source of energy for embryonic development.

Metabolic rate (J/mg/day) of developing eggs of *M. malcolmsonii* was calculated (Fig. 1). The eggs utilized about 0.79 J/mg/day during the early and late stages of development but only about 0.42 J/mg/day during mid-development. On recalculating the data reported for *M. lamarrei*, a similar trend was obtained (Fig. 1). It appears that the developing eggs require maximum energy during early (cleavage) and late (organogenesis) developmental stages.

M. malcolmsonii has an obligate but passive migrant larval stage (Kewalramani 1973). Since these larvae are transported to the estuary soon after hatching, they would need sufficient energy reserves to enable them to survive. The cumulative efficiency of utilization of various components during embryonic development of a few decapod crustaceans is presented in Table 2. *M. malcolmsonii* utilized dry matter, organic matter and fat more efficiently than other species. Table 3 shows the calorific content of eggs and larvae of four *Macrobrachium* species and their gross energy utilization efficiency and energy loss during development. *M. nobilii* and *M. malcolmsonii* utilized energy more efficiently and lost minimum energy on embryonic metabolism than other *Macrobrachium* species. Like *M. malcolmsonii, M. nobilii* is a riverine prawn and undertakes larval migration to the estuary to complete development (Balasundaram and Pandian 1982). The greater provision of reserve yolk energy in these larvae may be regarded as an adaptive feature for the larval migration.

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Table 1. *Macrobrachium malcolmsonii:* Average changes in chemical composition and calorific content of a single egg/larva. All weights in μg and percentage chemical compositions and energy density (KJ/g dry wt) in parentheses.

Parameter	Stage I	Stage II	Stage III	Stage IV
Live weight	60.90	62.80	66.72	92.50
Water	34.50 (56.70)	38.60 (61.50)	43.22 (64.80)	69.90 (75.60)
Dry weight	26.40	24.20	23.50	22.60
Ash	0.69 (2.61)	0.94 (3.90)	1.55 (6.60)	2.64 (11.70)
Organic substance	25.71 (97.40)	23.24 (96.03)	21.95 (93.40)	19.96 (88.32)
Protein	13.23 (50.11)	11.98 (49.50)	11.35 (48.30)	9.63 (42.61)
Fat	11.06 (41.90)	9.33 (38.64)	8.41 (35.80)	7.57 (33.50)
Carbohydrate	1.42 (5.40)	1.91 (7.90)	2.19 (9.32)	2.76 (12.21)
Energy (J/egg)	0.7371 (27.92)	0.6400 (26.47)	0.5900 (25.12)	0.5030 (22.24)

Table 2. Cumulative efficiency of utilization of various components of the yolk during embryogenesis of some decapod crustaceans.

	Percentage efficiency				
Species	Dry matter	Organic matter	Fat	Protein	Reference
Crangon crangon	70.3	65.7	33.6	83.0	Pandian (1967)
Homarus gammarus	81.8	70.0	47.4	87.7	Pandian (1970a)
Ligia oceanica	—	73.6	57.8	90.5	Pandian (1972)
Macrobrachium idella	61.5	—	26.6	47.6	Vijayaraghavan and Easterson (1974)
Macrobrachium lamarrei	72.9	69.9	51.7	91.6	Katre (1977)
Macrobrachium malcolmsonii	85.6	77.6	68.4	72.8	Present work

Table 3. Calorific contents of eggs and larvae of some *Macrobrachium* species during embryonic development (KJ/g dry wt). Gross efficiency and energy loss in percentage.

Species	Calorific content		Gross efficiency (GE)	Energy loss (EL)	Reference
	Eggs	Larvae			
Macrobrachium idella	26.08	18.46	44.0	56.0	Vijayaraghavan and Easterson (1974)
Macrobrachium lamarrei	26.51	23.96	66.0	34.0	Katre (1977)
Macrobrachium nobilii	29.39	24.08	73.7	26.3	Balasundram and Pandian (1982)
Macrobrachium malcolmsonii	27.92	22.24	68.2	31.8	Present work

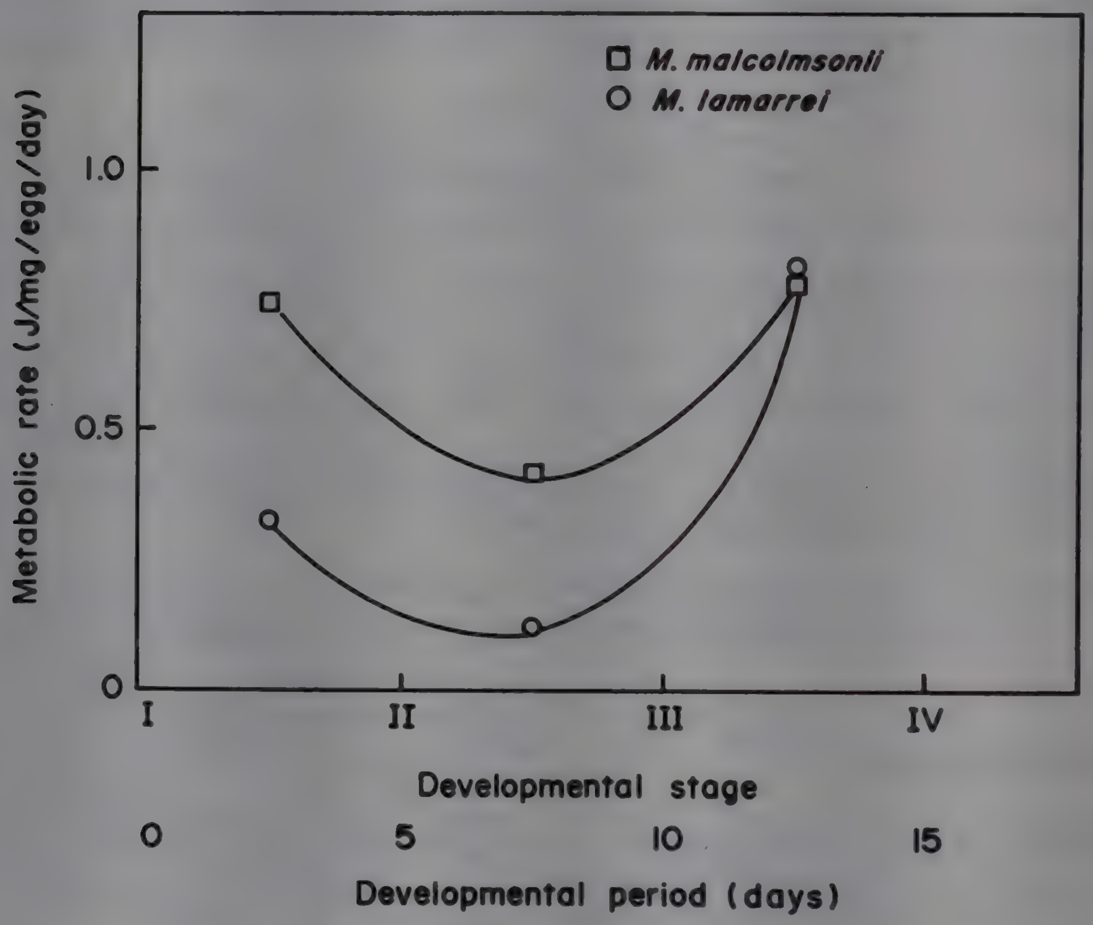

Fig. 1. Metabolic rate of *Macrobrachium* species at different developmental stages.

Egg Size and Larval Size Among Teleosts: Implications to Survival Potential*

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Abstract

A survey of the early life history characteristics of 135 teleost fishes from freshwater, marine, tropical, temperate and boreal habitats shows the influence of egg size and larval size on survival potential. Marine species have smaller eggs and larvae than freshwater species at similar temperatures. Coldwater species tend to have larger eggs and larvae than warmwater species. Egg diameters are positively correlated with larval lengths (Lh) and weights at hatching. The times from fertilization to onset of feeding (tf), to yolk and oil resorption (ty) and to irreversible starvation (ts), increase linearly with Lh and decrease exponentially with temperature. Both tf and ts are positive linear functions of ty. Thus, larger larvae with much yolk that lasts for a relatively longer period feed later, and if not fed, will starve later than small larvae with little yolk. Larger larvae will thus have the advantage under conditions of limited or variable food supply. Moreover, large larvae tend to have large mouths and are thus capable of ingesting large high-calorie prey. They also tend to have higher swimming speeds and greater potential to encounter food and avoid predators. There is no definite relation between growth rates and Lh, but tropical species with small eggs and larvae tend to have high growth rates. Survival potential has implications in the recruitment to natural stocks and in seed production in hatcheries.

Introduction

Among fishes, the survival potential at both the individual and the species level is greatly influenced by the parents, especially the female, in terms of egg size, fecundity, and conditions for incubation and early development as determined by reproductive behavior (Blaxter 1969; Hunter 1981). Species that simply broadcast their spawn into open waters often have high fecundity and small eggs (e.g., *Chanos)*, while species that show parental care often have relatively low fecundity and large eggs (e.g., *Oreochromis)*. Reproductive behavior affects survival in that the season and time of spawning, the spawning habitat, and the density of spawn determine the physicochemical environment (temperature, salinity, dissolved oxygen, pH, etc.) of the eggs and larvae, as well as their food and predators (Johannes 1978; Koski 1981; Blaxter and Hunter 1982; Lambert and Ware 1984). The inverse relation between fecundity and egg size must strike a balance with the environmental conditions encountered by the species (Blaxter 1969; Ware 1975).

While the effect of environmental conditions on the viability of spawn is duly recognized and well documented in the literature (e.g., Blaxter 1974; Lasker and Sherman 1981), the influence of egg size on survival potential has been relatively little studied (Blaxter and Hempel 1963; Bagenal 1971; Ware 1975; Wallace and Aasjord 1984).

Freshwater fishes like cyprinids, cichlids and salmonids are highly successful aquaculture species, whereas most marine fishes like siganids, serranids and sciaenids have been difficult to rear on a commercial scale. Perhaps the most striking difference between these two groups, which probably influences to a great extent their amenability to rearing in the hatchery, is the size of their respective eggs and larvae. Nash and Kuo (1975) contend that one factor that impedes the mass propagation of *Mugil cephalus* is its small eggs (0.93 mm) and larvae (2.6 mm), while the early success of culture techniques for *Pleuronectes platessa* and *Solea solea* can be attributed to their comparatively large eggs (1.6-2 mm) and larvae (3.5-6.5 mm). Events during the larval stage are generally acknowledged to be major determinants of recruitment success, and thus have important implications to capture fisheries as well.

In this paper, we review the literature and correlate several early life history characteristics with egg size and larval size, and show how such relationships affect survival potential, here taken to mean the ability to withstand starvation, to find food and to avoid predators during the larval stage. We collated information on 135 species of teleost fishes belonging to 50 families, most of which constitute the bulk of the production from the oceans, seas and inland waters. At least 135 publications were consulted, including Blaxter (1969), Shirota (1970), Russell (1976), Theilacker and Dorsey (1980), Hunter (1981), Philippart and Ruwet (1982), Moser et al. (1984), McGurk (1984), Bagarinao (1986) and the relevant references therein. Of the 135 species, 100 are marine and/or littoral and 35 are freshwater; 35 are boreal (inhabiting areas with, or have been reared at, water temperatures of < 10ºC), 43 are temperate (< 20ºC) and 57 are tropical (> 20ºC).

Egg Size and Larval Size

Most marine fishes, regardless of taxonomic affinities, demersal or pelagic habits, coastal or oceanic distributions, tropical or boreal ranges, spawn pelagic eggs that are fertilized externally and float individually near the sea surface (Moser et al. 1984). These eggs are generally spherical and range from about 0.5 mm to 4 mm in diameter, with a mode of about 1 mm. They hatch into relatively undeveloped larvae relying on yolk for nourishment while the organ systems develop to the point that they can feed on plankton. On the other hand, many littoral species and nearly all freshwater species lay demersal eggs that are generally much larger than the 1-mm mode of pelagic eggs and are frequently adhesive and laid in some sort of nest. In such fishes, larvae usually hatch at an advanced stage of development, often capable of early swimming and feeding. Parental care of eggs and larvae is observed in many of these species. In several fish groups, first-feeding larvae (e.g., *Sebastes*) or juveniles (e.g., *Poecilia*) are born, thus eliminating the highly vulnerable early larval stage.

Differences in diameters reflect true differences in yolk and caloric content (Blaxter and Hempel 1963; Kazakov 1981; Table 1, equations 1 and 2). A *Clupea harengus* egg weighs 150-300μg dry, depending on the race (Blaxter and Hempel 1963; the much larger *Salvelinus alpinus* weighs 16,000μg dry (Wallace and Aasjord 1984). A *Sardinops sagax* egg (56μg) contains about 0.3 cal (Lasker 1962), while the larger *M. saxatilis* egg (285-300μg) contains about 2.07-2.45 cal (Eldridge et al. 1981; Rogers and Westin 1981). Dry weight and caloric measurements are far from common in the literature on larval fishes.

Egg diameters (D) (mm) are correlated with larval lengths at hatching (Lh) (mm) (Fig. 1). For the species considered in this paper, the regression equation is Lh = 0.5731 + 2.3964 D (n = 135; r = 0.82). The correlation is improved if species with eggs having large perivitelline spaces (e.g., cyprinids and *Morone*) are left out of the computation (Table 1, equations 3 and 4).

The eggs and larvae of species living at low temperatures are generally larger than those at high temperatures (Fig. 1B-1C; Table 1, equations 5 to 8). Freshwater species tend to have larger eggs and larvae than marine species at similar temperatures. Tropical marine fishes (e.g., *Siganus*) tend to have the smallest eggs and larvae, and boreal freshwater species (e.g., *Oncorhynchus*) tend to have the largest. It is probably significant that the most successful aquaculture species (e.g., cyprinids and cichlids) are in tropical freshwaters, while the world's great fisheries center on coldwater marine fishes (e.g., *Clupea, Engraulis, Mallotus*).

In addition to the marked interspecific differences, egg size and larval size also vary intraspecifically. Blaxter and Hempel (1963) found egg weight variation of 65% among six races of herring. In many species, such as tilapias (Peters 1963), salmonids (Gall 1974; Kazakov 1981), and *Morone* (Rogers and Westin 1981), egg size is related to the size and age of the spawners. Seasonality in egg size has been observed in many species, with larger eggs being produced in the local winter and spring, and smaller ones in the local summer and autumn (Bagenal 1971; Blaxter and Hunter 1982). Larval lengths at hatching can vary with temperature and salinity within species; and the trends of such variation differ among species (Laurence and Rogers 1976).

Correlates of Size

The lengths of newly hatched larvae are highly correlated with weight (Fig. 2A, Table 1, equations 9 and 10), which is a measure of the amount of metabolizable endogenous energy (in terms of yolk, oil globule and body tissues) available to the larvae before they start external feeding and/or before the onset of irreversible starvation. The close correlation of lengths to weights at hatching makes it reasonable to use the former in species comparisons.

The times (days) from fertilization to onset of feeding tf (when the eyes are fully pigmented and the mouth open), yolk and oil globule resorption (ty), and irreversible starvation (ts) are directly related to Lh (Table 1, equations 11-13). Like egg size and larval size, tf, ty and ts are negative exponential functions of temperature (T) (equations 14-16). Fig. 3 shows plots of ty against Lh and T, which are similar to those for tf and ts.

In turn, tf and ts are both strongly correlated with ty (Table 1, equations 17 and 18). Thus, larger larvae which have much yolk that lasts a longer time start to feed later, and if not fed, starve later than smaller larvae with less yolk. The ratio tf/ty has a mean value of 0.70 ± 0.18 (n = 94), while the ratio ts/ty has a mean value of 0.83 ± 0.30 (n = 31). McGurk (1984) has made similar observations as above.

Mouth size at onset of feeding is directly correlated with length at hatching (Fig. 2B; Table 1, equation 19). Mouth size sets the upper limit for prey that can be ingested. In general, smaller larvae can take only small prey like phytoplankton, while larger larvae can take larger prey like copepods at first feeding (Shirota 1970; Hunter 1981). Larvae of several piscivorous species (e.g., *Seriola* and *Katsuwonus)* have disproportionately large mouths (Fig. 2B).

Larval length at first feeding (Lf) is a function of both D and Lh: $Lf = 4D$ and $Lf = 1.357\ Lh$ (Shirota 1970). The advantage of a longer body in larvae is primarily improvement in locomotion (Blaxter and Staines 1971; Hunter 1972, 1981; Braum 1978). The swimming speed of larvae at the time of first feeding is directly related to larval length, and the volume of water searched for food increases exponentially with both larval length and swimming speed (Table 1, equations 20-22).

Growth rates have no significant relation to larval length at hatching nor to mouth size, but increase exponentially with temperature among species (Table 1, equations 23-25). Fast growth is one way that fish larvae can escape the high predation rates during the larval stage (Ware 1975; Houde and Schekter 1981). Small tropical larvae tend to have high growth rates (Table 1, equation 23), perhaps to compensate for the initial size disadvantage (Bagarinao 1986). Shirota showed that larvae with large mouths tend to have high growth rates since they can ingest large, high-calorie prey. Obviously, growth is variously influenced by innate and external factors.

Specific Studies

In the classical study on *Clupea harengus*, Blaxter and Hempel (1963) showed that larvae from stocks with large eggs (8-9 mm vs. 5-7 mm) were longer at hatching, had larger yolk sacs, were longer at the time of yolk resorption and lived longer after yolk was exhausted. In *Trachurus symmetricus*, even a small difference in egg diameter was shown to be significant: larvae from 1-mm eggs starved more slowly, lived longer without food and grew twice as fast as those from 0.9 mm eggs (Theilacker 1981). Noting that *Gadus morhua* had lower total mortality and larger larvae (4.5 mm) at hatching than *Melanogrammus aeglefinus* (3.5 mm) at all salinity-temperature combinations, Laurence and Rogers (1976) concluded that the former may have a better chance of survival than the latter. Further studies showed that the two species were virtually identical in comparative metabolism, feeding ability and growth (Laurence 1978). Studying tropical marine species, Houde (1974) and Houde and Schekter (1981) did not find large differences in growth and survival performance among *Anchoa mitchilli*, *Archosargus rhomboidalis* and *Achirus lineatus*, although *A. rhomboidalis* had greater survival and growth potential at low food concentrations. These three species have similar-sized eggs (0.75-0.8 mm) and larvae (1.9-2.3 mm), with Wh = 18, 28, 22 g, respectively. Among three other tropical marine species reared in the hatchery *(Chanos chanos*, *Lates calcarifer* and *Siganus guttatus)*, *S. guttatus* is the most difficult to rear through the first week, and has the smallest eggs and larvae, earliest yolk resorption, the smallest mouth, but the highest yolk conversion ratio and initial growth rate (Bagarinao 1986). The other two species are easy to rear; *C. chanos* has larger eggs and larvae, but *L. calcarifer* has a large long-lasting oil globule and earlier onset of feeding.

Egg size effects on survival potential have consistently emerged from studies on salmonids (Bagenal 1969; Fowler 1972; Kazakov 1981). In *Salmo gairdneri*, differences in progeny performance associated with differences in age of females can be accounted for by the variation in egg sizes: larger eggs have higher hatchability and produce larger faster growing fingerlings to 75 days (Gall 1974). *Salvelinus alpinus* larvae from large eggs were larger, had larger yolk sacs, grew more quickly and suffered less initial mortality than those from small eggs (Wallace and Aasjord 1984). In *Oncorhynchus keta*, early stock fry emerge from the gravel 12-13 days later and weigh 35 mg less than late stock fry, but measure about the same, 38.7 mm (Koski 1981). Similarity in length of fry at emergence in these two stocks and for *O. keta* throughout its range indicates strong selective pressure: length of fry affects swimming performance and vulnerability to predation in this species (Koski 1981). This study on *O. keta* demonstrates the interaction of the physical incubation environment (particularly gravel composition) and the parental characteristics in determining fry quality and survival.

There are few studies on other freshwater species. Balon (1977), Philippart and Ruwet (1982) and Noakes and Balon (1982) showed that eggs and larvae of nest-guarding *Tilapia* species are smaller than those of mouthbrooding *Oreochromis*, *Sarotherodon* and *Labeotropheus* species, and consider that mouthbrooding is an advanced reproductive style that insures high survival to the juvenile stage. Egg size effects on survival of cyprinid larvae do not seem to have been addressed yet, although the aquarium fish trade attests to the relative ease of propagation of the various cyprinid species.

Among species reared at similar temperatures (15-20°C), there are specific differences in survival potential. While *Sardinops sagax* larvae ($Wh = 36\ \mu g$) incur an energy deficit before they are capable of feeding (Lasker 1962), *Micropterus salmoides* larvae (Wh = 280μg) do not do so at any time before complete yolk resorption (Laurence 1969). *Morone saxatilis* larvae (tissue dry weight at hatching 42μg, Wh > 42μg) survive for about 30 days from hatching when not given food at all, do not have a well defined point of no return, and tolerate delays in inital feeding of up to 16 days with 70% survival; in this species, the large oil globule which contributes 55% of egg weight but 72% of total egg energy lasts more than 18 days (Eldridge *et al.* 1981; Rogers and Westin 1981). *Leuresthes tenuis* larvae (Wh = 362μg) also do not exhibit a critical period at the time of yolk resorption, and regardless of how long initial feeding is delayed (up to 20 days after hatching), 80% or more of previously unfed larvae begin feeding when food is made available (May 1971). In contrast, *Engraulis mordax* larvae (Wh = 21μg) starve irreversibly when feeding is delayed more than 4.6 days after hatching (Hunter 1981). Effects of egg size on offspring fitness may also be seen intraspecifically, as in *Etheostoma spectabile* (Marsh 1986).

Selection for Optimum Egg Size

Any life history characteristic, such as egg size and larval size, that affects survival potential is subject to natural selection. From an ecological viewpoint, Ware (1975) argued that variations in egg size and growth rates of fish have evolved to increase the probability of individual survival, on the average, and hence maximize recruitment to the adult stock. Optimal egg size represents a balance between mass and numbers so that enough eggs survive the incubation period and so that individual larvae grow fast enough within the limits of their food supply. In a model, he showed that at low temperatures and longer or variable incubation periods, large eggs have the advantage: the larger resulting larvae are less vulnerable to predation and will pass the larval stage in a shorter time despite the slower growth rate. At high temperatures, the selective advantage shifts to small eggs because the higher fecundity compensates for the high predation rate and the faster growth offsets the initial small size of the larvae. Jones and Hall (1974) hypothesized that egg size *(*in *Gadus* and *Melanogrammus)* is selected such that the newly hatched larvae are an appropriate size to feed on young growing stages of plankton. Thus, under conditions where cohorts of copepods comprise sizes and stages ("feeding range") small enough for small larvae to initially feed on, egg size would be small. With a wider feeding range, large eggs would be selected.

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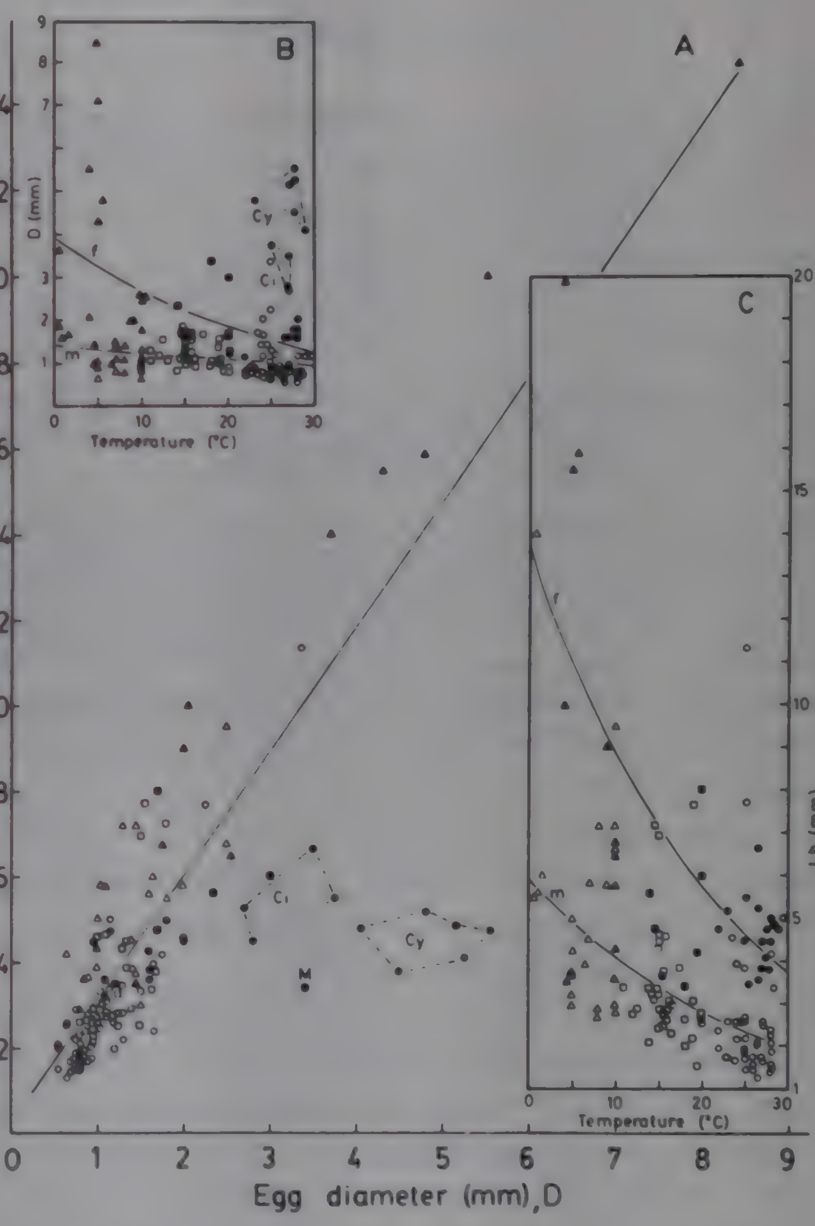

Fig. 1. A, relation between diameter of fertilized egg and length of larvae at hatching. Drawn curve (r = 0.90, n = 128 species) excludes points M and Cy in polygon. B and C, the negative exponential relation of temperature to D and Lh differs for marine and freshwater species (m, f curves). The f curve in B excludes points in the dashed polygons Ci and Cy. M, Morone; Cy, cyprinids; Ci, cichlids. Data for Ci are maximum egg lengths; data for Cy are diameters of water hardened eggs with very wide perivitelline space around a yolk mass 1.2-1.5 mm in diameter. Circles, tropical species; squares, temperate; triangles, boreal. Open points, marine; closed, freshwater.

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*SEAFDEC Aquaculture Department
Contribution No. 175 and ICLARM Contribution No. 350.

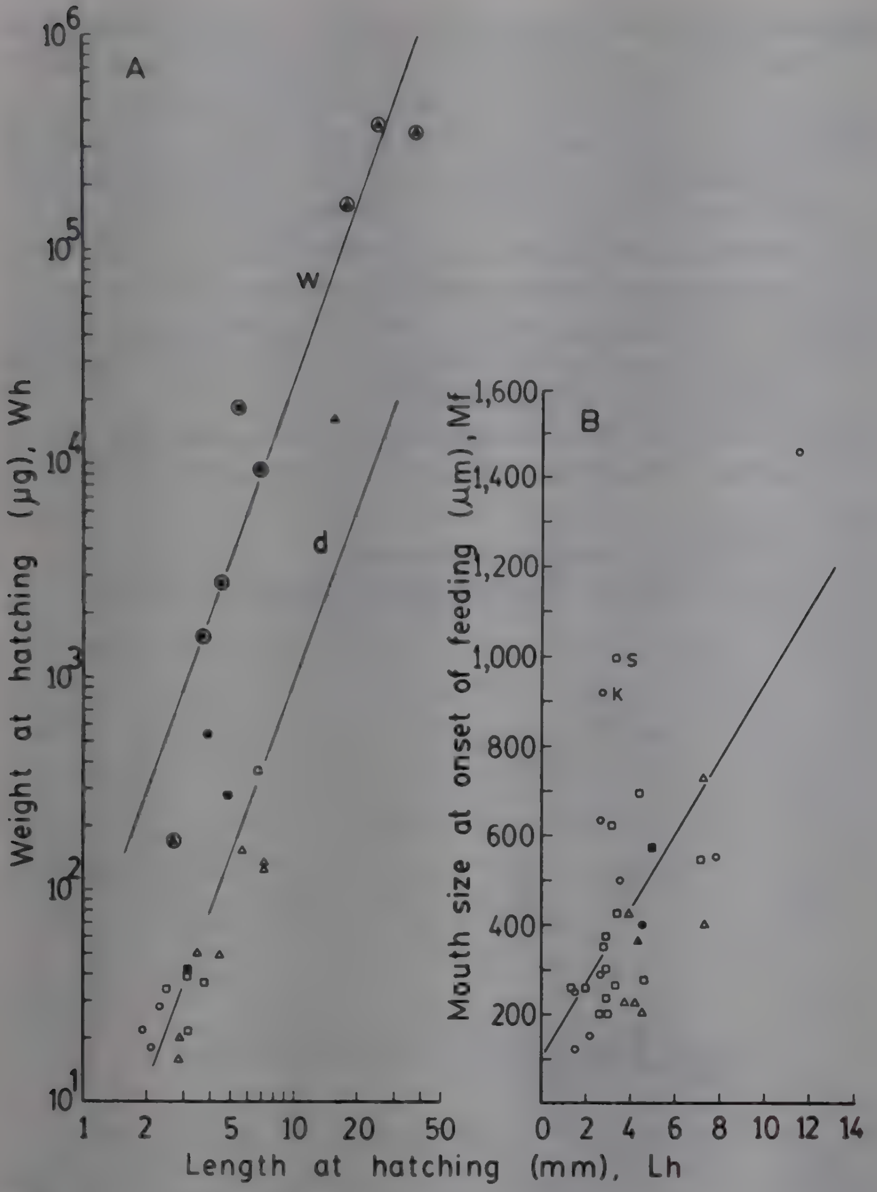

Fig. 2. A, power regressions of larval length on dry weight (d curve, r = 0.86, n = 19) and wet weight (w curve, r = 0.96, n = 8) at hatching. Most wet weight values are for cichlids and salmonids. B, mouth size at onset of feeding is correlated with length at hatching (r = 0.62, n = 33); most data points from Shirota (1970); S, *Seriola;* K, ***Katsuwonus.*** Circles, tropical species; squares, temperate; triangles, boreal. Open points, marine; closed, freshwater.

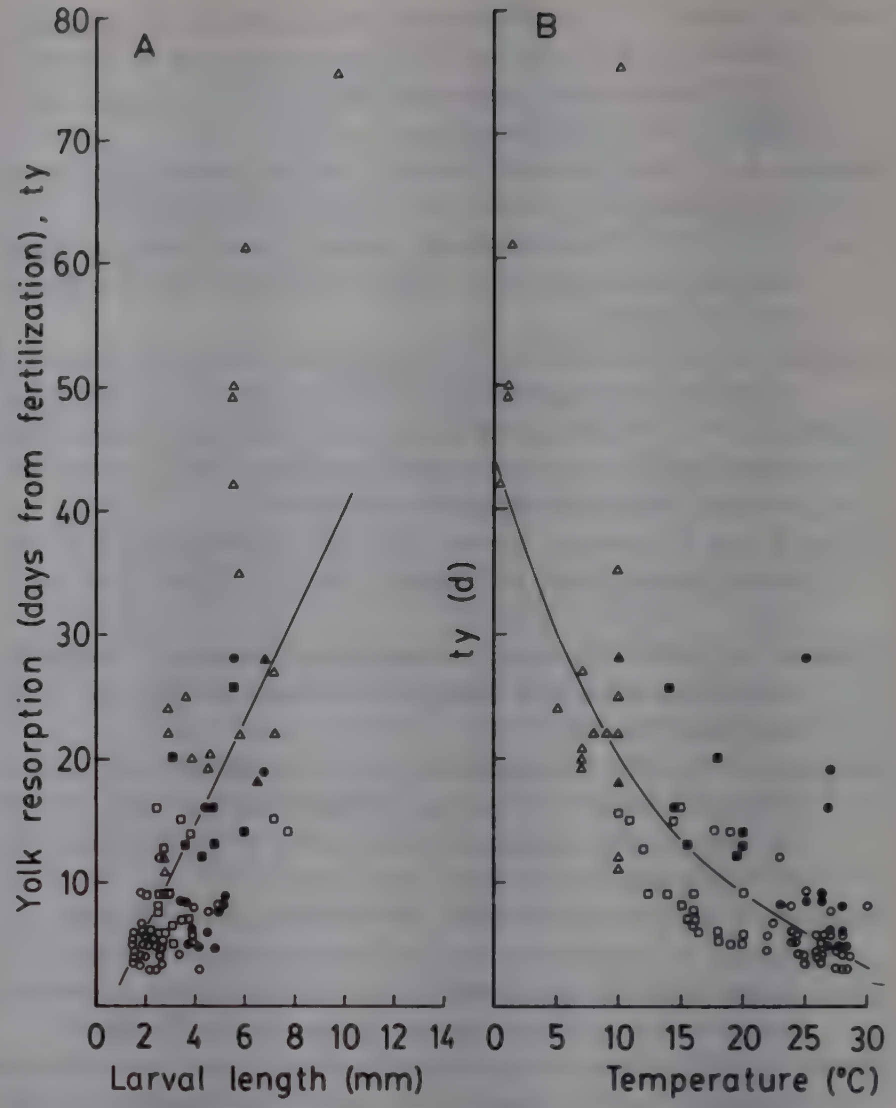

Fig. 3. A, time to yolk resorption increases with length at hatching (r = 0.68, n = 96), but B, exponentially decreases with temperature (r = 0.78). Circles, tropical species; squares, temperate; triangles, boreal. Open points, marine; closed, freshwater.

Table 1. Regression equations for relationships discussed in this paper. Except those of equations 23 and 25, all r values are significant (equation 1, $p < 0.05$; others, $p < 0.01$).

Equation no.	Regression equations	r	n
1	log We = 1.5924 + 3.0809 log D	0.74	8
2	log E = −2.5079 + 1.1210 log We	0.98	6
3	Lh = 0.5731 + 2.3964 D	0.82	135
4	Lh = 0.2634 + 2.8316 D	0.90	128*
5	ln D, m = 0.3517 − 0.0141 T	−0.31	99
6	ln D, f = 1.3607 − 0.0388 T	−0.56	25**
7	ln Lh, m = 1.7639 − 0.0355 T	−0.57	99
8	ln Lh, f = 2.6153 − 0.0461 T	−0.67	35
9	log Wh, d = 0.2678 + 2.7085 log Lh	0.86	19
10	log Wh, w = 1.6568 + 2.7131 log Lh	0.96	8
11	tf = −3.4868 + 3.6124 Lh	0.67	94
12	ty = −2.5036 + 4.2521 Lh	0.68	96
13	ts = −1.6084 + 4.3468 Lh	0.70	30
14	ln tf = 3.7144 − 0.0939 T	−0.80	94
15	ln ty = 3.7449 − 0.0778 T	−0.78	96
16	ln ts = 3.8470 − 0.0834 T	−0.86	30
17	tf = −1.0412 + 0.7807 ty	0.99	94
18	ts = −0.3783 + 1.2371 ty	0.93	31
19	Mf = 99.2298 + 82.9410 Lh	0.62	33
20	S = −1.9866 + 1.4592 Lf	0.78	12
21	ln V = −2.4601 + 0.3906 Lf	0.86	9
22	ln V = −2.4815 + 0.2973 S	0.94	6
23	ln G = −0.6736 − 0.1002 Lh	−0.22	52
24	ln G = −2.2834 + 0.0672 T	0.71	52
25	G = 0.5510 − 0.0003 Mf	−0.27	12

We, egg dry weight; Wh, larval weight at hatching (µg); D, egg diameter; Lh, length at hatching; Lf, length at first feeding (mm); E, egg energy content (cal); T, temperature (°C); Mf, mouth size at onset of feeding (µm); tf, time from fertilization to onset of feeding; ty, time to yolk resorption; ts, time to irreversible starvation (days); S, swimming speed (mm/s); V, volume searched for food (l/hr); G, growth rate during larval stage (mm/d); m, marine; f, freshwater; d, dry; w, wet. Data for equation (19) mostly from Shirota (1970), plus others. Data for equation (20) similar to those tabulated by Theilacker and Dorsey (1980), with additions.

*Excludes cyprinids and *Morone* in Fig. 1A.

**Excludes cyprinids and cichlids in Fig. 1B.

Effects of Ammonia and Nitrite on Larval Development of the Shrimp *Penaeus monodon*

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Abstract

The water in the hatchery pond during the development of *Penaeus monodon* larvae was sampled and frozen every day after the eggs hatched. The number of the larvae was counted and the water was sampled when they metamorphosed from nauplius to the 12th day of the postlarval stage. The sampled water was thawed at room temperature and analyzed for pH, salinity, ammonium-N and NO_2-N. NH_3-N was calculated from ammonium-N based on Whitfield's theory. Fourteen ponds stocked with nauplii served as test groups, and two ponds which contained only water served as controls. In the control groups, ammonium-N and NO_2-N were 12.1-74.9 μg/l and 0.3-5.4 μg/l, respectively. In the test groups, ammonium-N and NO_2-N were 7.5-808.4 μg/l and 0.8-118.1 μg/l, respectively. The correlation of NO_2-N with survival rate was more significant than that of ammonium-N with survival rate. The survival rate (Y as %) had an exponential regression with average NO_2-N level (X as μg/l): $Y = 74.2341\ e^{-0.1607x}$. The ponds which had higher than 20% survival rate on the final day had slightly declined pH levels. Therefore, monitoring the levels of ionized and un-ionized ammonia and controlling the levels of NO_2-N and pH would result in better survival of larval prawn.

Introduction

In Taiwan, artificial propagation of the tiger shrimp *Penaeus monodon* succeeded in 1968 (Liao et al. 1969). Induced maturation with eyestalk ablation was widely applied by shrimp hatchery farmers resulting in the rapid development of the industry in recent years. There are more than 1,200 hatcheries with a production of 1.5 billion juveniles of *P. monodon*. Survival rate of larval shrimp metamorphosed from nauplius to postlarva on the 12th day (PL_{12}) is less than 20% (Chen et al. 1985; Liao 1984). Several factors, mainly water quality, feed organisms and health of gravid females are considered to affect survival rate. During the metamorphosis of larval shrimp, the input of feed organisms and the excretion of organisms cause the deterioration of water quality. This paper deals with the relationship of ammonia and nitrite with the survival rate of larval shrimp during metamorphosis.

Materials and Methods

Underground seawater was pumped from the coast adjacent to the hatchery. The water was filtered with a 200-mesh net before entering the hatchery ponds (4 x 7 m) and aerated with a blower for one day. Table 1 shows the water parameters. Shrimp eggs hatched 18 hours after spawning. The nauplii were transferred to the hatchery ponds at stocking density of 100,000/tonne. Water temperature of 28-30°C and salinity of 33-35 ppt were maintained while the water was aerated continuously. The number of larvae was estimated by counting samples of water from left, middle and right sides. Two runs with fourteen batches were carried out as test groups and two ponds without shrimp but with water only, served as control (Table 2). One liter was sampled from each pond every afternoon with PVC bottle and frozen for later analysis until the larvae attained PL_{12}. The dark-room style hatchery in which the nauplii were reared in darkness through all the stages was adopted (Liao 1984). As the larval shrimp metamorphosed to zoea stage I (Z_1), they were fed filament diatom cells, *Skeletonema*, and as they reached mysis stage 2 (M_2), they were fed newly hatched artemia nauplii. Every three hours, the health of the larval prawn was checked with the aid of battery flashlight. When the water turned too turbid, one-third of the water was pumped out through a 50-mesh net and the same volume of water was let in in the morning. No drug or artificial feed was administered.

The frozen water samples were carried to the laboratory and thawed at room temperature and filtered with Toyo 5C paper. The pH was measured with a Photovolt 112 pH meter and the salinity determined by a Beckman RS7-B salinometer. Ammonia-N (including un-ionized and ionized form) was measured by the phenolhypochlorite method (Solorzano 1969) and the un-ionized ammonia-N (NH_3-N) calculated from the Bower-

Bidwell's equation based on Whitfield's theory (Whitfield 1974; Bower and Bidwell 1978). Nitrite-N (NO_2-N) was measured with the method described by Bendschreider and Robinson (1952).

Results and Discussions

The concentrations of ionized ammonia, NH_3-N and NO_2-N were in the range of 12.1-74.9 μg/l, 1.4-9.5 μg/l and 0.5-5.4 μg/l in the control groups and in the range of 7.5-808.4 μg/l, 0.7-79.7 μg/l and 0.8-118.1 μg/l in the test groups. In the 14 test ponds, two had a survival rate less than 10%, four between 10 and 20%, five between 20 and 30% and three more than 30% in the final day (Figs. 1-4). Ammonia is the principal end product of protein catabolism excreted by crustaceans (Campbell 1973; Kinne 1976). The un-ionized NH_3 is toxic to fish but NH_4^+ has little or no toxicity (Wuhrmann and Worker 1948; Hemens 1966; Brown 1968). The portion of NH_3 depends primarily on pH, temperature and to a lesser extent salinity (Bower and Bidwell 1978).

Fig. 1 shows that both G2 and H2 ponds had increasing concentrations of ammonium-N, NH_3-N and NO_2-N. In G2 pond, ammonium-N increased to 808.4 μg/l and NO_2-N increased to 118.1 μg/l on the 21st day the survival was only 4% in spite of water exchange at 9th and 16th days. No postlarva survived in the H2 pond where the ammonium-N changed from 25.3 to 269.1 μg/l and NO_2-N changed from 0.8 to 78.3 μg/l at the 18th day, although one-third of the water volume was changed on days 7, 9, 12 and 15. Ammonium-N, NH_3-N and NO_2-N increased immediately the next day after the water was changed. Ammonium-N and NH_3-N in H2 were significantly lower than those in G2 pond. NO_2-N in G2 remained lower than 40 μg/l up to day 16, whereas NO_2-N in H2 increased to more than 40 μg/l on day 8. This suggests that NO_2-N had more influence than ammonium-N and NH_3-N.

Fig. 2 shows data in the ponds where the shrimp had 10-20% survival on the final day. Ammonium-N ranged from 40.4 to 537.4 μg/l, NH_3-N from 1.2 to 42.8 μg/l and NO_2-N from 1.7 to 36.2 μg/l.

Fig. 3 shows data in the ponds where the shrimp had 20-30% survival on the final day. The ammonium-N, NH_3-N and NO_2-N also increased but pH readings seemed to decline as the larvae developed. Ammonium-N ranged from 24.5 to 701 μg/l, NH_3-N from 2.3 to 40.6 μg/l and NO_2-N from 0.9 to 18.9 μg/l.

Fig. 4 shows the ponds where the shrimp had more than 30% of survival on the final day. Ammonium-N ranged from 20.8 to 738.2 μg/l and NH_3-N from 2.1 to 42.6 μg/l. The NO_2-N ranged from 0.9 to 13.1 μg/l and was below 10 μg/l most of time while the pH readings also declined slightly.

The accumulation of ammonia is a serious problem in closed system culture (Campbell 1973). The NH_3-N in the ponds ranged from 3.2 to 30.3 μg/l in 0% survival, 0.7 to 79.7 μg/l in 4% survival, 1.2 to 42.8 g/l in 10-20% survival, 2.3 to 40.6 μg/l in 20-30% survival and 2.1 to 42.6 μg/l in more than 30% survival (Figs. 1-4). All these showed that the concentration of NH_3-N was far below the 0.4 to 2.31 mg/l of 96-hour LC_{50} values for crustaceans (Colt and Armstrong 1981) and far below the 1.29 mg/l of 48-hour LC_{50} to seven penaeid species larvae (Wickins 1976). The NH_3-N level which reduced the growth by 50% of that of control was 220 μg/l for *P. semisulcatus*, 370 μg/l for *P. japonicus*, 400 μg/l for *P. occidentalis*, 590 μg/l for *P. setiferus* and 690 μg/l for *P. schmitti*. A maximum acceptable level which reduced growth by 1-2% of that of the control was estimated to be 100 μg/l NH_3-N for penaeid larvae (Wickins 1976). No scientific data are available on the acute and long-term effects of NH_3-N on *P. monodon* larvae. Delistraty et al. (1977) stated that the incipient LC_{50} value and safe value of NH_3-N for larvae of American lobster, *Homarus americanus*, were 1.4 mg/l and 140 μg/l, respectively. Jayasankar and Muthu (1983a) reported that the 24-hour LC_{50} values of NH_3-N for nauplius, protozoea and mysis of *P. indicus* were 290 μg/l, 950 μg/l and 3.17 mg/l, respectively; the NH_3-N level that reduced the growth by 50% of that of control was 250 μg/l for *P. indicus* larvae. In this study, the concentration of ammonium-N increased gradually but was not higher than the safe level of 1.2 mg/l ammonia-N or 93 μg/l NH_3-N which was a safe level for rearing *P. indicus* larvae suggested by Jayasankar and Muthu (1983a).

During metamorphosis, the ponds were aerated continuously and DO concentration was always kept at 5.1-5.7 mg/l. Ammonia is oxidized through nitrification even at DO concentrations of 0.5-1.0 mg/l (Stenstrom and Poduska 1980). The 48-hour LC_{50} of NO_2-N to postlarvae of seven penaeid species was 170 mg/l (Wickins 1976). A level of 6.4 mg/l NO_2-N caused 50% reduction in the growth of *P. indicus* larvae after 34 days. The 96-hour LC_{50} value of NO_2-N to *Macrobrachium rosenbergii* 10-14 day-old larvae was 8.6 mg/l and a level of 1.8 mg/l NO_2-N caused 35% reduction in the growth of *M. rosenbergii* larvae after eight days (Armstrong et al. 1976). There are no reports on the acute and chronic effect of NO_2-N on *P. monodon* larvae. The conversion of hemoglobin to methemoglobin in the presence of NO_2-N with the subsequent loss of oxygen-binding capabilities has been documented (Smith and Williams 1974; Smith and Russo 1975). This may result in hypoxia and cyanosis (Kiese 1974). It is likely that the same reaction may occur within the copper of crustacean hemocyanin. Mevel and Chamroux (1981) reared the *P. japonicus* (14.5 and 1.01 g) in a closed system and observed that the shrimp seemed to be sensitive to concentrations of NO_2-N higher than 100 μg/l.

Nitrate and nitrite are the products of nitrification and the release of hydrogen ions lowers the pH in closed systems (Wickins 1976; Sharma and Ahlert 1977). The ponds where the larvae had more than 20% survival on the final day had declining pH, suggesting the establishment of nitrification, although the pH was not lower than 6.45. Wickins (1976) demonstrated that the larvae of *P. monodon* in a recirculated water system were not affected by values as low as pH 6.45. Although nitrite was also highly toxic to aquatic organisms, nitrite levels in this study were considerably below the 8.5 to 15.4 mg/l NO_2-N of 96-hour LC_{50} on shrimps (Colt and Armstrong 1981) and below the level of 1.8 mg/l NO_2-N which caused 35% reduction in the growth of *M. rosenbergii* (Armstrong et al. 1976), below the level of 6.4 mg/l NO_2-N which caused 50% reduction in the growth of penaeid larvae (Wickins 1976). They were also and below the safe level of 0.18 mg/l NO_2-N for *P. indicus* (Jayasankar and Muthu 1983b).

Statistical analysis indicated that the survival on the final day had a decaying exponential relationship with average NO_2-N (Fig. 5):

$$Y = 74.2341\, e^{-0.1607x}$$

where Y is survival rate on the final day and x is the average NO_2-N level (μg/l). Manthe et al. (1984) demonstrated that nitrite was the most toxic form of nitrogen to the molting blue crab (10-15 cm in width). This proved that the NO_2-N level was more significant than ammonium-N and NH_3-N levels in correlation with survival in ponds.

Regardless of the ammonium-N and NH_3-N levels, when NO_2-N increases to 15 μg/l, >30% shrimp survival cannot be expected; similarly as NO_2-N increases to 25 μg/l, no more than 20% survival can be expected and at 78 μg/l, no more than 10% survival. Therefore, frequent monitoring of the levels of ammonium-N and NH_3-N and controlling the levels of pH and NO_2-N would give better better survival of larval shrimp in hatcheries.

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Table 1 Water parameters in the hatchery

pH		8.24	±	0.12	
Turbidity	(NTU)	0.9	±	0.5	
Dissolved oxygen	(mg/l)	5.4	±	0.3	
Ammonium-N	(μg/l)	48.4	±	19.2	
NO_2-N	(μg/l)	2.0	±	0.8	
NO_3-N	(μg/l)	68.7	±	62.8	
PO_4-P	(μg/l)	3.8	±	1.6	
Sulfate	(mg/l)	3,642	±	419	
Sulfide	(μg/l)	23.2	±	4.9	
Total alkalinity	(mg/l)	70	±	1	as $CaCO_3$
Total hardness	(mg/l)	4,503	±	232	as $CaCO_3$
Zn	(μg/l)	2	–	21	
Pb	(μg/l)	nd	–	10	
Cd	(μg/l)	nd	–	24	
Cu	(μg/l)	nd	–	86	
Ni	(μg/l)	nd	–	21	
Fe	(μg/l)	nd	–	22	
Mn	(μg/l)	nd	–	10	
Cr	(μg/l)	nd			
Co	(μg/l)	nd			
Hg	(μg/l)	nd			

Table 2. Parameters of seawater in hatchery ponds where larval prawn was reared from nauplius to the 12th day of the postlarval stage and survival rate on the final day.

Pond	Duration	Survival rate	pH Range	pH Average	Ammonium-N (μg/l) Range	Ammonium-N (μg/l) Average	NH_3-N (μg/l) Range	NH_3-N (μg/l) Average	NO_2-N (μg/l) Range	NO_2-N (μg/l) Average
H2	12/5-12/22 1984	0	8.12–8.42	8.29 ± 0.07	25.3 – 269.1	122.0 ± 82.4	3.2 – 30.3	12.9 ± 9.1	0.8 – 78.3	34.7 ± 25.2
G2	12/1-12/21 1984	4	8.12–8.34	8.24 ± 0.06	7.5 – 808.4	261.5 ± 227.4	0.7 – 79.7	28.4 ± 24.8	0.9 –118.1	24.8 ± 28.6
G3	3/15-4/5 1985	12.5	7.91–8.30	8.08 ± 0.11	117.2 – 537.4	295.4 ± 122.0	6.7 – 37.1	22.1 ± 9.2	8.7 – 36.2	15.8 ± 6.9
G5	3/15-4/5 1985	13	7.94–8.26	8.10 ± 0.10	40.4 – 449.8	189.8 ± 114.3	1.2 – 25.1	12.8 ± 6.7	3.3 – 22.3	8.1 ± 5.4
G7	12/2-12/23 1984	17.9	7.92–8.28	8.11 ± 0.10	57.1 – 515.0	221.8 ± 150.7	3.3 – 31.8	14.8 ± 9.7	1.7 – 18.4	5.9 ± 4.8
G4	3/15-4/5 1985	20	7.90–8.28	8.05 ± 0.10	145.6 – 537.4	282.1 ± 119.8	8.3 – 42.8	18.7 ± 9.4	6.3 – 25.6	10.9 ± 5.4
G10	12/5-12/26 1984	21.5	7.98–8.30	8.14 ± 0.09	43.1 – 474.3	203.2 ± 103.0	4.4 – 34.0	15.9 ± 7.6	1.8 – 13.9	5.9 ± 3.4
G7	3/15-4/5 1985	23	7.94–8.28	8.09 ± 0.10	31.0 – 621.7	247.4 ± 168.5	2.3 – 32.4	16.0 ± 9.9	3.4 – 12.8	7.1 ± 2.2
G4	12/1-12/20 1984	23.8	7.94–8.38	8.12 ± 0.15	24.5 – 698.3	246.5 ± 229.4	3.1 – 37.0	14.9 ± 11.5	0.9 – 13.6	5.2 ± 3.1
G6	12/1-12/22 1984	25	7.90–8.26	8.06 ± 0.15	47.4 – 671.7	337.6 ± 217.8	4.2 – 40.6	19.4 ± 10.5	1.3 – 18.9	8.5 ± 5.7
G9	12/5-12/26 1984	25	7.98–8.31	8.16 ± 0.09	51.1 – 367.6	207.7 ± 90.3	5.3 – 30.4	16.1 ± 8.0	1.7 – 11.2	5.7 ± 2.4
G5	12/1-12/20 1984	32.1	7.84–8.36	8.07 ± 0.16	23.2 – 738.2	309.8 ± 268.3	2.1 – 42.6	17.4 ± 13.9	0.9 – 13.1	5.9 ± 3.9
H3	12/8-12/29 1984	33.3	8.00–8.40	8.17 ± 0.13	49.8 – 546.4	204.2 ± 134.3	3.6 – 32.9	15.4 ± 7.7	1.4 – 8.7	4.6 ± 2.2
G3	12/1-12/20 1984	36	7.88–8.36	8.07 ± 0.16	20.8 – 731.8	289.5 ± 255.8	2.4 – 38.6	15.5 ± 11.6	1.3 – 10.0	5.5 ± 2.7
G1	12/1-12/22 1984	–(Control)	8.06–8.20	8.15 ± 0.05	16.0 – 74.9	38.9 ± 14.4	1.4 – 9.5	3.9 ± 2.3	0.5 – 3.0	1.3 ± 0.8
G1	3/15-4/5 1985	–(Control)	8.12–8.24	8.18 ± 0.04	12.1 – 42.6	22.8 ± 8.7	1.1 – 4.4	2.3 ± 0.9	3.1 – 5.4	4.1 ± 0.9

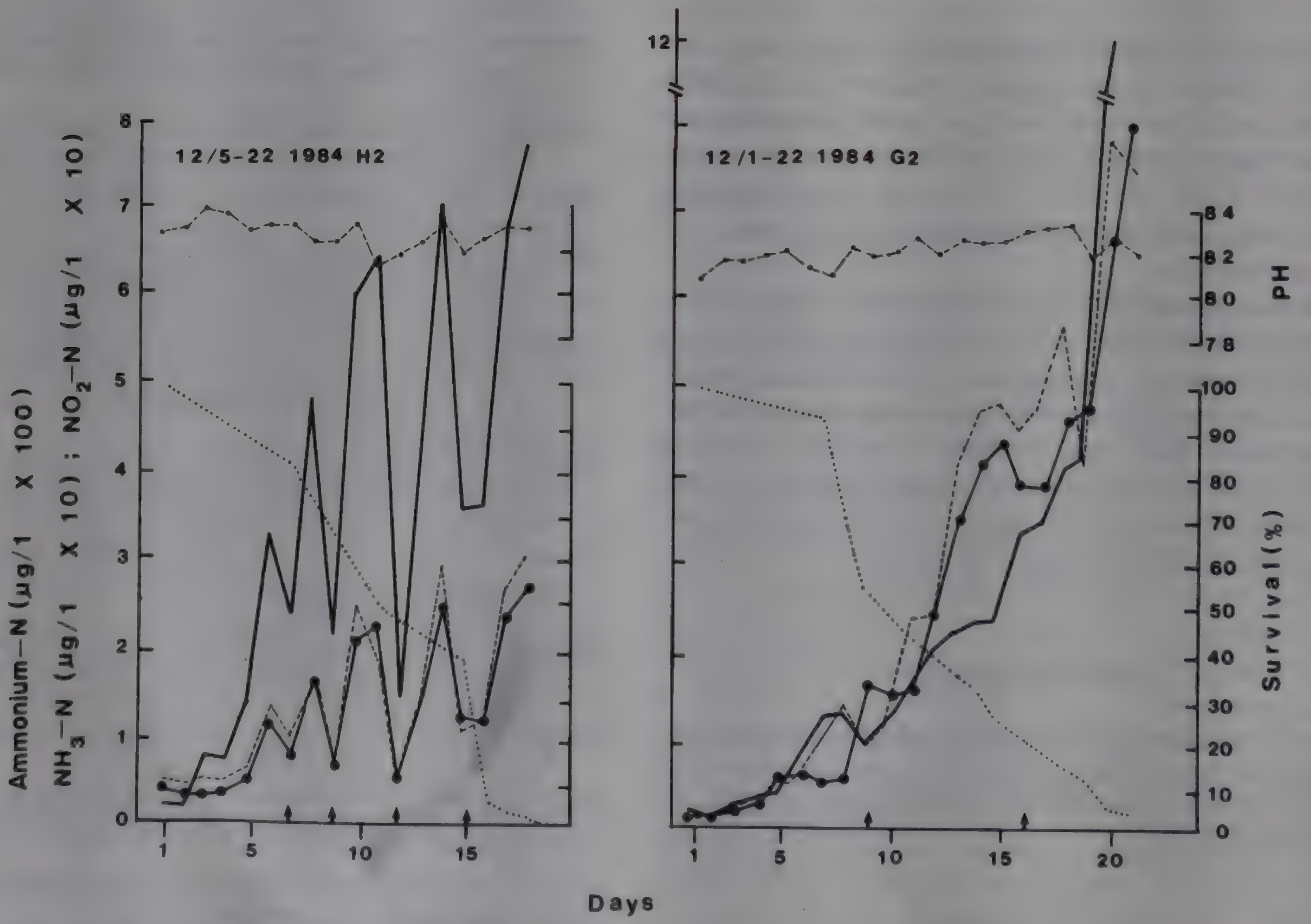

Fig. 1. Fluctuation of pH, ammonium-N, NH_3-N and NO_2-N and survival of larval prawn from nauplius to pL. •-•-•-•: pH, ●——●: ammonium-N, ----------: NH_3-N, ———: NO_2-N, ··········: survival. ↑: The day water was exchanged by 1/3 V.

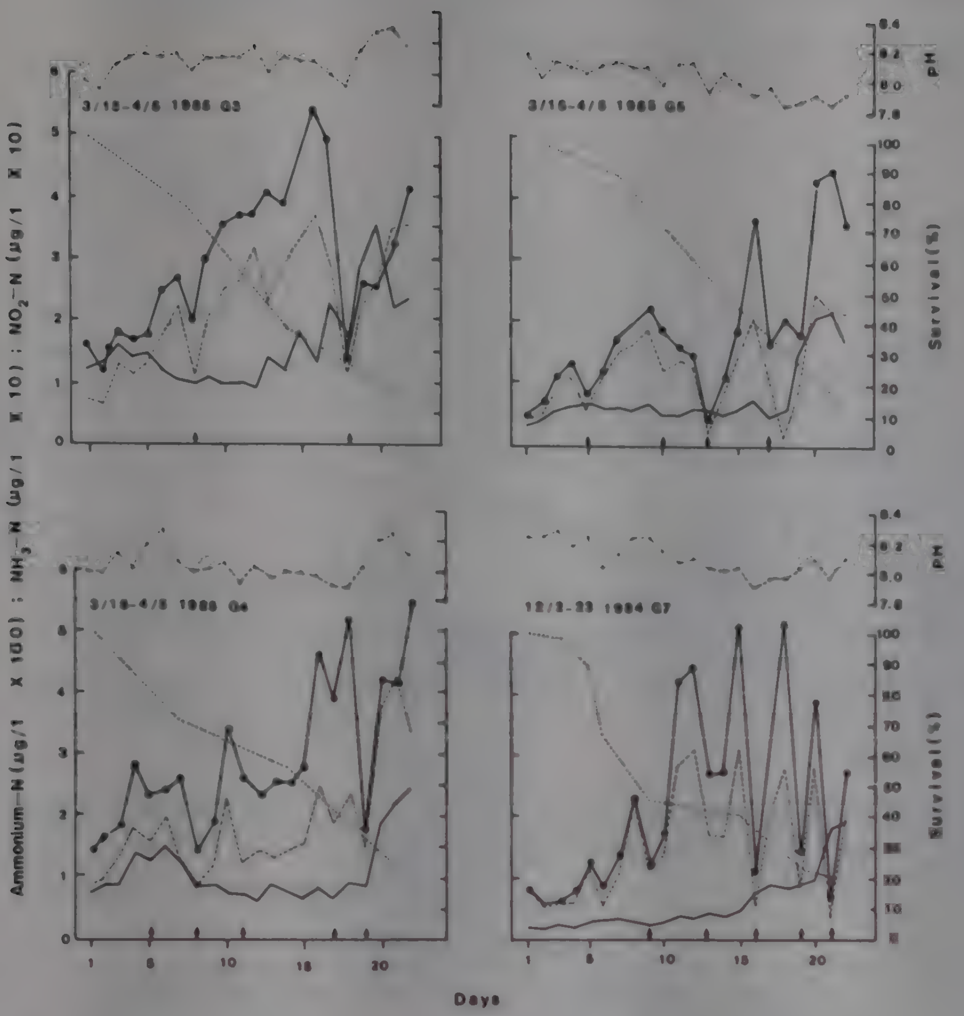

Fig. 2. Fluctuation of pH, ammonium-N, NH_3-N and NO_2-N and survival of larval prawn from nauplius to pL. •–•–•–•: pH, ●———●: ammonium-N, ———: NH_3-N, ———: NO_2-N, ...: survival. ↑: The day water was exchanged by 1/3 V.

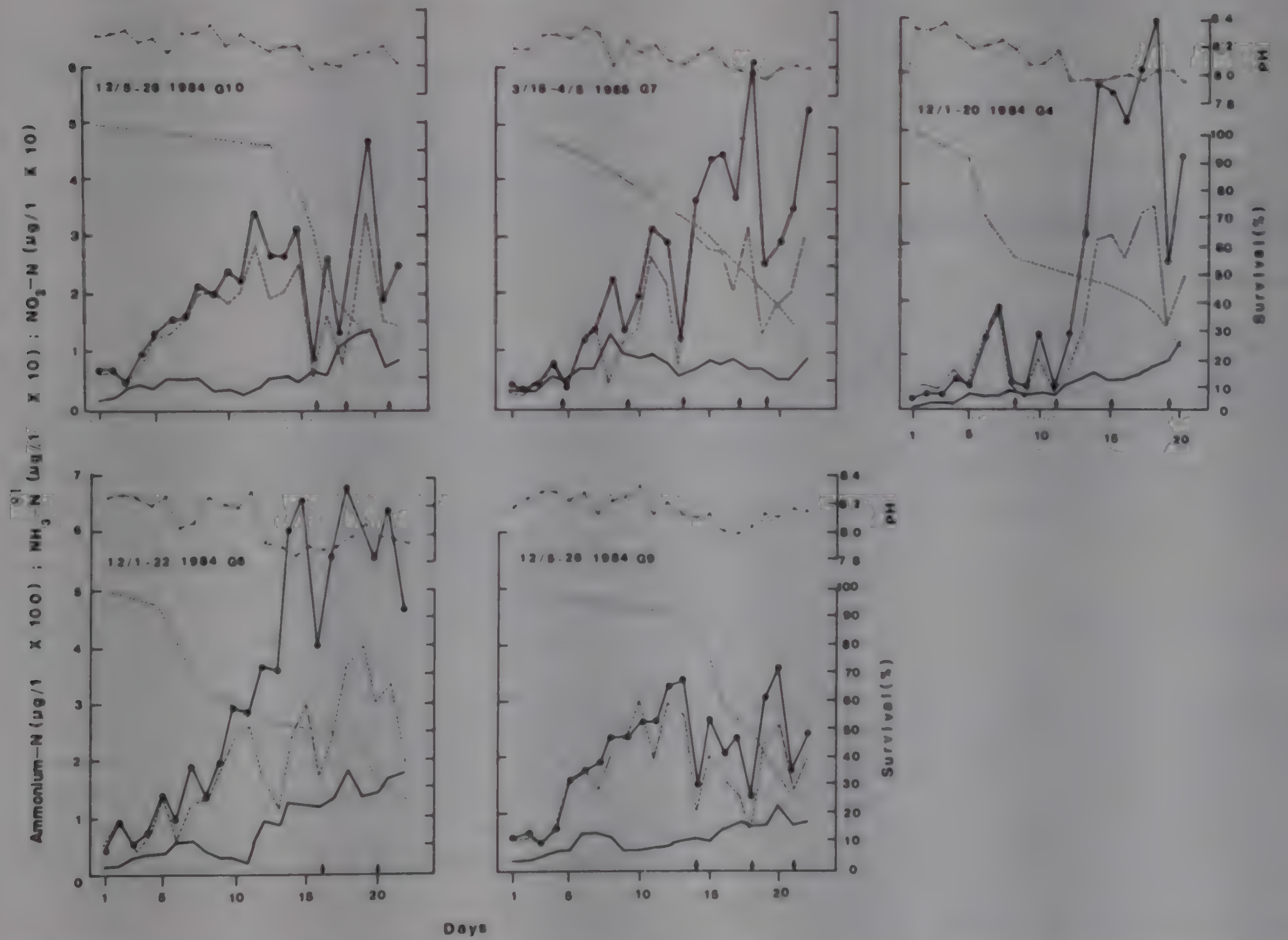

Fig. 3. Fluctuation of pH, ammonium-N, NH_3-N and NO_2-N and survival of larval prawn from nauplius to pL. •–•–•–•: pH, ●———●: ammonium-N, -------: NH_3-N, ———: NO_2-N, ...: survival. ↑: The day water was exchanged by 1/3 V.

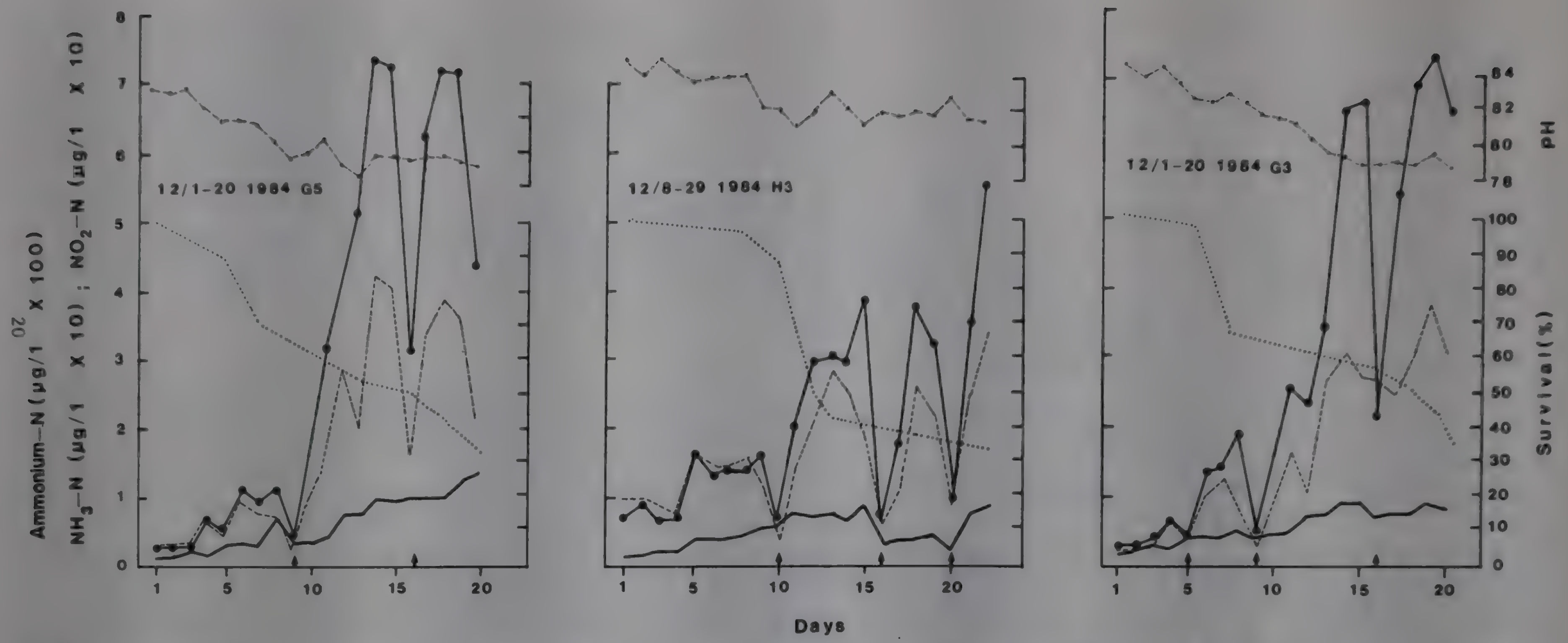

Fig. 4. Fluctuation of pH, ammonium-N, NH_3-N and NO_2-N and survival of larval prawn from nauplius to pL. •-•-•-•: pH, ●———●: ammonium-N, ------------: NH_3-N, ————: NO_2-N, ···········: survival. ↑: The day water was exchanged by 1/3 V.

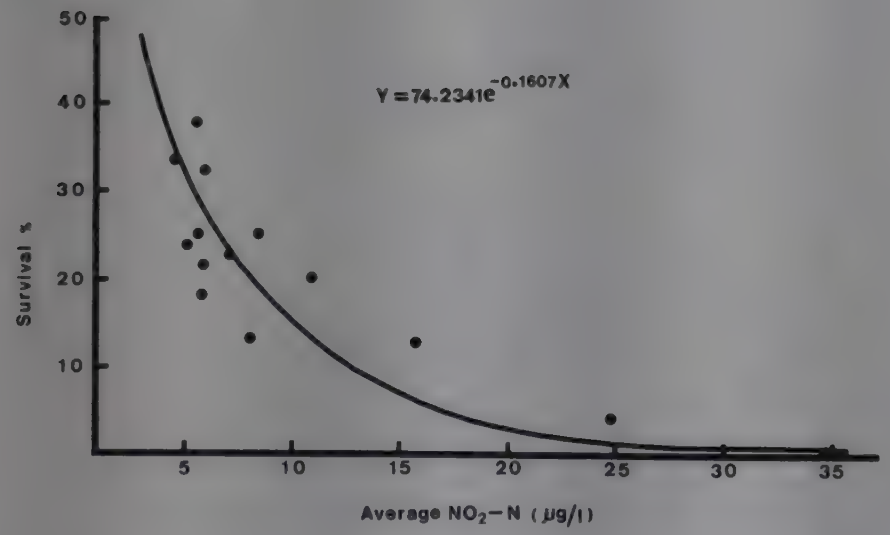

Fig. 5. The relationship of survival rate at final day nauplius metamorphosed to pL 12 and the average NO_2-N (µg/l) level of each pond.

Induced Spawning and Larval Rearing of Grouper (*Epinephelus salmoides* Maxwell)

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Abstract

Broodfish of grouper *(Epinephelus salmoides)* were induced to spawn by hormonal induction. The hormones used for this experiment were HCG + pituitary gland (PG) and LRH-a. The results showed that at dosages of 500 IU HCG + 3 mg of PG per kg of fish for first injection and 1,000 IU HCG + 3 mg of PG per kg of fish at the final injection at an interval of 24 hours, the treated fish spawned naturally in a spawning tank 12 hours after the final injection. At lower dosages of 500 IU HCG + 3 mg PG at 12-hour intervals or 500 IU HCG + 3 mg PG at 24-hour intervals or using 10 mg LRH-a at 12-hour intervals, the eggs can be artificially fertilized only by stripping.

The larval rearing experiment was conducted in a 250-l fiberglass tank at the stocking density of 2,500 larvae per tank. Feeds used in this experiment were: (a) *Isochrysis* + sea urchin eggs; (b) *Isochrysis* + *Brachionus;* and (c) *Tetraselmis* + *Brachionus*. The results showed that newly hatched larvae fed with *Isochrysis* mixed with sea urchin eggs and then *Brachionus* from day 10 had the best survival rate (9% at day 30).

Introduction

The grouper *(Epinephelus salmoides)* is a popular marine food fish of high market value in Southeast Asia. It has been farmed commercially in marine cages and ponds in Thailand, Malaysia, Singapore, Hongkong and China. The fish can grow fast and attain market size of 600-800 g in about 6-8 months (Chua and Teng 1980; Chen 1979). The fry used for culture are usually obtained from the wild which means the supply is very erratic and inconsistent. Large-scale commercial production of grouper in marine cages or ponds is, therefore, hampered by shortages of fry. To ensure consistent and adequate supply of fry, efforts have been made to produce them under controlled conditions.

The grouper undergoes sex reversal starting out as a female when young and then becoming male when older and larger. Sex determination of 68 specimens of *Epinephelus tauvina* collected from the South China Sea showed the biological minimal size of females to be 450-500 mm, while male fish with ripe testes were above 700 mm in standard body length and more than 11 kg in body weight (Tan and Tan 1974). Red grouper *(E. morio)* become more significantly male at the weight of 9 kg and above (Moe 1969). In fact, male grouper are scarce and found only in deep seas. Breeding of grouper, therefore, depends on the availability of males. Female *Epinephelus tauvina* can be sexually transformed with methyl testosterone treatment. Females can also be induced to ovulate by application of human chorionic gonadotropin (HCG) and salmon pituitary extract (Chen 1979). However, the survival rate of fry was very low.

The purpose of this paper is to report the results of the experiments on induced spawning of grouper using different types of hormones and larval rearing systems.

Materials and Methods

The grouper *(E. salmoides)* used were caught in bamboo traps and were transported to the experimental station by boat. The size of fish ranged from 3 to 15 kg. They were conditioned in 60-t rectangular concrete tanks for 6-12 months at stocking density of 1/2 m^3, fed daily with fresh trashfish at the rate of 5% body weight; 50-70% of the water in the tanks were changed daily.

Egg maturation was determined through egg diameter measurement. The fish were first anaesthetized.

The eggs were sampled using a 2-mm plastic tube inserted through the genital opening. The maturation of the males was determined by pressing the abdomen for milt. Only females with mean egg diameter of 400μm and males with running milt were selected for induced spawning by hormone injections. The induced fish were placed in separate cages installed in another holding tank.

The different types of hormone used, dosages and time intervals between the first and second injection are shown in Table 1. Twelve females with weights ranging from 3.6 to 6.5 kg and four males ranging from 10 to 16 kg were selected for this study. The female and male fish were injected intramuscularly with the assigned hormone and dosage as shown in Table 1. In cases where ovulation did not occur after the second injection, a third injection was given.

The fertilized eggs from natural spawning in the spawning tanks were collected with a fine dip net (100μm mesh size). Other planktonic organisms and detritus included in the collection were removed by screening. Unfertilized eggs which settled to the bottom of the tanks were removed by siphoning. The eggs were placed in hatching containers and hatched out in about 15-20 hours.

The larvae from the hatching containers were collected and stocked in nine 250-l fiberglass larval rearing tanks at a stocking rate of 2,500 larvae per tank. Three types of feed (Fig. 1) were tested in three replicates in a completely randomized design.

The larvae were reared indoors under intensive care conditions. Beginning on day 3 the feed was introduced. During the larval rearing trial, 20-30% of the water was changed daily.

Sea urchins were collected from the sea and kept alive in tanks with flow-through seawater. The gonads were collected 1 hour before feeding time and immersed in filtered seawater. The eggs were removed by opening the gonad and collected by means of a screen net. Only eggs were used for feeding and they were given to the larvae twice daily at 0800 hr after water management and at 1600 hr.

Results

The fish under treatment D (Table 1) spawned naturally in the spawning tank 12 hours after the final injection (Table 2). Fish under treatment B (Table 1) ovulated 12-15 hours after the final injection, but fertilization was completed only by artificial stripping. The fish in treatments A and C required a third injection for ovulation. The eggs were artificially fertilized 9-15 hours after the final injection.

Ovulation rate of the females treated with hormonal injections at 24-hour intervals was higher than those at 12-hour intervals (Table 2). This may be due to the handling which caused the fish stress.

The results of the larval rearing experiment showed that sea urchin eggs are a suitable feed for grouper larvae. The diameter of the sea urchin eggs was about 50μm. The survival rate of fry fed with sea urchin eggs and *Isochrysis* from hatching to 20-days old was 9%, and was 2% for those fed with *Isochrysis* and *Brachionus*. None of the fry fed with *Tetraselmis* and *Brachionus* survived; all the larvae died after a culture period of six days (Table 3).

Discussion

Two interesting observations were made. First, the grouper, *E. salmoides*, can be spawned naturally in captivity after hormone manipulation. This is very advantageous for mass production of grouper fry as it minimizes the mortality of the spawners through stress due to handling and stripping. Second, sea urchin eggs have been shown to be a suitable food for the grouper larvae.

The most suitable time interval between the first and second injection is 24 hours. The shorter period might have caused the fish more stress damage. The dosages of hormones used, 500 IU HCG + 3 mg PG/kg of fish, and double the quantities for the second injection, showed the best result. However, the appearance of natural spawning in captivity after hormone manipulation showed the possibility of induced spawning by environmental manipulation which occurs in seabass, *Lates calcarifer*. The key to successful spawning by environmental manipulation lies in the condition of the broodstock. Broodstock development should be the prime work for further studies.

The fish larvae which were fed with *Tetraselmis* and *Brachionus* died six days after stocking, despite the fact that *Brachionus* is one of the most valuable larval feeds. This might be attributed to the size of the *Brachionus* and activity of the *Tetraselmis*. It should be noted that there are two types of *Brachionus*: L type (100-150μm) and S type (40-100μm). Unfortunately, the predominant type in the hatchery was L type, and since it is larger than the larvae's mouth this may be the cause of their mortality. Studies on feeding grouper larvae with S type *Brachionus* should be done in the future.

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Table 1. Type of hormones, dosages and time intervals used for induced spawning of *E. salmoides.*

Treatment	Hormone used	Dosage	Time interval (hr)
A	HCG + pituitary gland of Chinese carp	500 IU + 3 mg PG/kg	12
B	HCG + pituitary gland of Chinese carp	500 IU + 3 mg PG/kg	24
C	LRH-a	10 μg/kg	12
D	HCG + pituitary gland of Chinese carp	(1st) 500 IU + 3 mg PG/kg (2nd) 1,000 IU + 3 mg PG/kg	24

Table 2. Induced spawning of *Epinephelus salmoides* using different hormones.

Treatment	Fish number	Body weight (kg)	Time interval (hr)	Number of injections	Hormone used	Remarks
A*	1	4.5	12	3	HCG 500 IU + 3 mg PG/kg of fish	Partial ovulation, 12 hr after final injection. No fertilization of eggs.
	2	3.6	12	3		Partial ovulation. No fertilization.
	3	5.2	12	3		Partial ovulation, 12 hr after final injection; fertilization rate 40%; hatching rate 20%. Larvae died after 6 days.
	4	4.8	12	2		Partial ovulation, 15 hr after final injection. Fertilization rate 30% but no hatching.
B*	5	4.2	24	2	HCG 500 IU + 3 mg of fish	Ovulation 12 hr after final injection. Fertilization rate 60%; hatching rate 30%; larvae healthy.
	6	6.0	24	2		Partial ovulation. No fertilization.
	7	5.8	24	2		Ovulation, 15 hr after final injection. Fertilization rate 30%; hatching rate 60%; larvae died after 10 days.
C*	8	4.1	12	3	LRH-a 10 μg/kg of fish	Partial ovulation, 15 hr after final injection. Fertilization rate 40%; hatching rate 50%; larvae healthy.
	9	4.5	12	3		Ovulation 12 hr after final injection. Fertilization rate 80%; hatching rate 40%; larvae healthy.
D**	10	5.2	24	2	1st injection – HCG 500 IU + 3 mg PG/kg of fish. 2nd injection – HCG 1,000 IU + 3 mg PG/kg of fish.	All fish spawned naturally in spawning tank 12 hr after final injection. Fertilization rate 30%; hatching rate 70%, larvae very healthy.
	11	5.5	24	2		
	12	6.5	24	2		

*Insufficient mature males.

**Used running ripe male only with one injection given during the last injection for the female.

Table 3. Survival of *Epinephelus salmoides* larvae at day 20. Tanks started with 2,500 fish and fed according to treatments in Fig. 1.

Treatment	Tank no.	No. of fish at day 20	Survival (%)
1	3	0	0
	5	0	0
	9	0	0
2	1	14	0.56
	4	6	0.24
	8	52	2.08
	Average	24	0.96
3	2	172	6.88
	6	288	11.52
	1	219	8.76
	Average	226.3	9.05

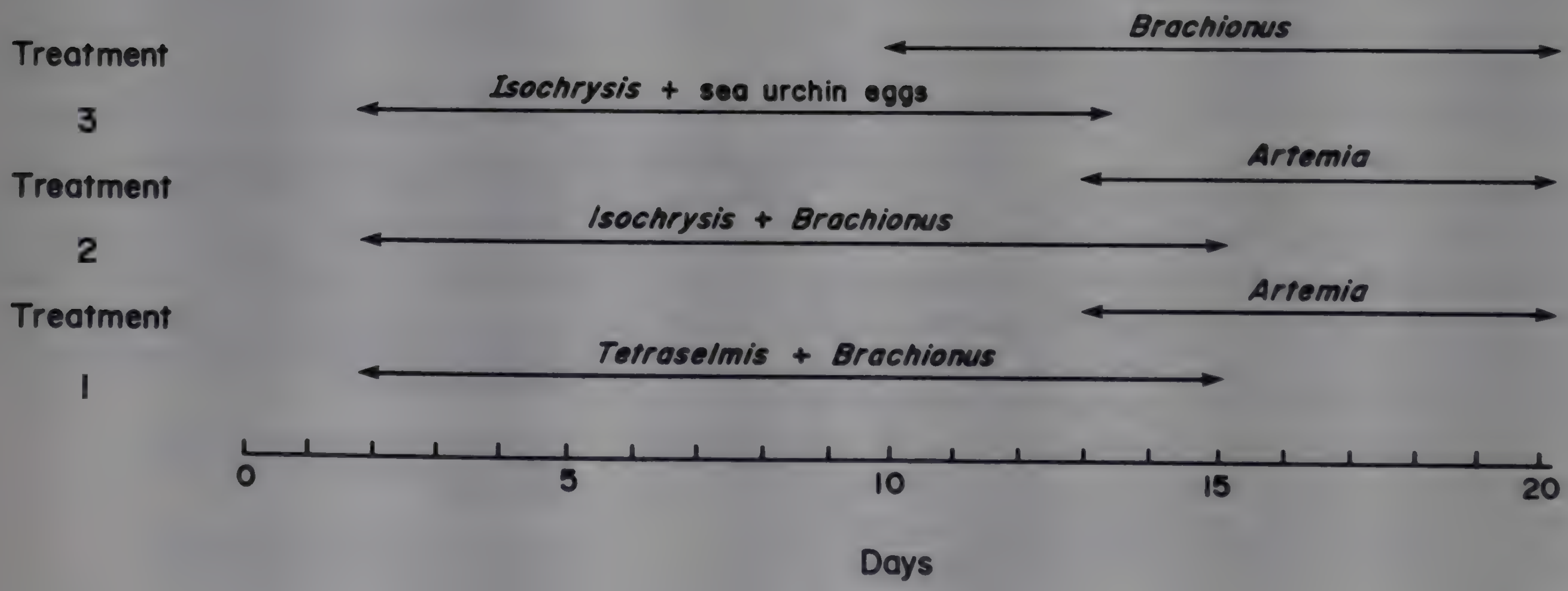
Treatment 3
Brachionus
Isochrysis + sea urchin eggs
Treatment 2
Artemia
Isochrysis + Brachionus
Treatment 1
Artemia
Tetraselmis + Brachionus
0
5
10
15
20
Days

Fig. 1. Feeding scheme for larval rearing of *Epinephelus salmoides*.

Induction of Gonadotropin Secretion and Ovulation in Teleosts Using LHRH Analogs and Catecholaminergic Drugs: A Review

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LIN, H.R. and R.E. PETER. 1986. Induction of gonadotropin secretion and ovulation in teleosts using LHRH analogs and catecholaminergic drugs: a review, p. 667-670. *In* J.L. Maclean, L.B. Dizon and L.V. Hosillos (eds.) The First Asian Fisheries Forum. Asian Fisheries Society, Manila, Philippines.

Abstract

Gonadotropin (GtH) release in teleosts is regulated by the stimulatory effect of GtH-releasing hormone (GnRH) and the inhibitory effect of a GtH release-inhibitory factor (GRIF). Dopamine acts as a GRIF directly at the level of the pituitary to modulate spontaneous GtH release and to modulate the actions of GnRH in goldfish and all other teleosts investigated. The intensity of the dopamine inhibitory tone differs among species, and, within a species, the intensity may also change during the reproduction cycle. The removal of the endogenous dopamine inhibition and an increase in GnRH stimulation of GtH secretion are required for the rapid increase in blood GtH levels that normally triggers ovulation. Injection of D-Ala^6 Pro^9-N ethylamide - LHRH (LHRH-A) or some other analog of LHRH is effective in stimulating GtH secretion in teleosts; however, in salmonids, the injection of LHRH-A alone is relatively ineffective in stimulating ovulation. Notably, injection of teleosts with a dopamine antagonist drug, such as pimozide, or drugs that cause depletion or block synthesis of dopamine, such as reserpine, greatly potentiate the GtH-releasing activity of LHRH-A. The combined treatment of pimozide or reserpine with LHRH-A represents a highly effective means of inducing the ovulation and spawning of farmed fishes.

Nature of Gonadotropin-Releasing Hormones (GnRH)

Studies on a number of teleosts have demonstrated the presence of GnRH in crude hypothalamic extracts (for review see Peter 1983). The primary structure of the predominant GnRH in chum salmon has been determined to be (Trp^7, Leu^8)-luteinizing hormone-releasing hormone (Sherwood et al. 1983). More recently, Sherwood et al. (1984) and Breton et al. (1984) have demonstrated that this form of GnRH is present in the brain of a wide range of teleost species.

Evidence for a Gonadotropin Releasing-Inhibitory Factor (GRIF)

Peter et al. (1978) found that large lesions in the ventrobasal hypothalamus of pre-ovulatory female goldfish caused a dramatic prolonged increase in serum GtH levels and ovulation. These results appear to indicate the presence of a GtH release-inhibitory factor (GRIF) and the abolition of GRIF that allows spontaneous release of GtH. On the basis of a series of brain-lesioning experiments on goldfish, Peter and Paulencu (1980) suggested that GRIF originates from the anterior-ventral nucleus preopticus periventricularis (NPP), in the region of the preoptic recess, and that a pathway courses from this center to the pituitary. Additional evidence for the presence of GRIF comes from studies in which the pars distalis of the pituitary was transplanted from one goldfish to another at various locations (Peter et al. 1984). Such a transplanted pars distalis releases GtH spontaneously, irrespective of location of the transplant, indicating that GtH secretion is normally under tonic inhibition. Pars distalis transplants beside the brain released more GtH than those transplanted into the brain ventricles, indicating the presence of GRIF in the brain.

Dopamine as GRIF

The involvement of catecholamines in the neuroendocrine regulation of GtH release in the goldfish was first demonstrated by Chang et al. (1983). Long-term treatment with 6-hydroxydopamine, as well as a single intraperitoneal injection of reserpine, increased levels of circulating GtH. Intraperitoneal injection of alpha-methyl-para-tyrosine or carbidopa, drugs known to block L-dopa and dopamine synthesis respectively, also elevated serum GtH levels. Injections of diethyl-dithio-carabamate, an agent capable of inhibiting the conversion of dopamine into norepinephrine, had no effect on circulating GtH concentrations. These suggest that dopamine may have an inhitory effect on GtH secretion in goldfish. This is supported by findings that intraperitoneal injection of dopamine and the dopamine receptor agonist apomorphine decreased whereas intraperitoneal injections of the dopamine antagonists pimozide and metoclopramide elevated serum GtH concentrations (Chang and Peter 1983a, 1983b; Chang et al. 1984b; Sokolowska et al. 1984).

Evidence for the direct action of dopamine on the goldfish pituitary to inhibit spontaneous GtH release was provided by *in vitro* and *in vivo* studies. Goldfish pituitary fragments and dispersed cells, in a column perifusion system, have a high rate of spontaneous GtH release (Chang et al. 1984c). Addition of dopamine to the perifusate reduced the level of GtH release from dispersed goldfish pituitary cells as well as pituitary fragments. Goldfish in which the anterior preoptic region has been lesioned have increased serum GtH levels due to abolition of endogenous GRIF allowing a high rate of spontaneous GtH secretion (Peter and Paulencu 1980). Intraperitoneal injection of either dopamine or apomorphine significantly depressed the lesion-induced increase in serum GtH levels (Chang and Peter 1983a). Pars distalis transplants have a high spontaneous GtH release rate, causing marked increases in circulating GtH levels (Peter et al. 1984); intraperitoneal injection of dopamine or apomorphine significantly reduced the highly elevated serum GtH values in fish bearing pars distalis transplants (Chang et al. 1984a).

Dopamine also inhibits the GnRH-stimulated GtH release in goldfish. Intraperitoneal injection of dopamine or apomorphine reduced the magnitude of the increase in serum GtH levels stimulated by Des-Gly^{10} (D-Ala^{6})-LHRH ethylamide (LHRH-A) on a dose-dependent basis (Chang and Peter 1983a; Chang et al. 1984b). Dopamine completely blocked the LHRH-A stimulation of GtH release *in vitro* (Chang et al. 1984c). On the other hand, injections of the dopamine antagonists, pimozide or metoclopramide, facilitated the LHRH-A-induced increase in serum GtH levels (Chang et al. 1984b; Sokolowska et al. 1984, 1985a, 1985b; Peter et al. 1985).

Intraperitoneal injection of reserpine, 6-hydroxydopamine, alpha-methyl-para-tyrosine, and carbidopa all potentiated the GtH-releasing activity of LHRH-A in goldfish; however, injection of diethyl-dithio-carbamate did not influence the responsiveness to LHRH-A (Peter et al. 1986). These results indicate that drugs which block the synthesis of dopamine or cause depletion of dopamine from presynaptic terminals also influence the responsiveness of goldfish to injected LHRH-A. This lends further support to the idea that dopamine serves as GRIF in goldfish.

Induced Ovulation with GnRH Agonists and Dopamine Antagonists

In sexually mature female goldfish, injection of the dopamine antagonist pimozide not only facilitates the action of LHRH-A in stimulating GtH secretion but the treatment combined with pimozide and LHRH-A can also stimulate increases in blood levels of GtH similar to, or higher than, those observed during spontaneous ovulation, and induce a high rate of ovulation (Chang and Peter 1983b; Chang et al. 1984b; Sokolowska et al. 1984, 1985a, 1985b). The ovulatory response in the goldfish is probably dependent on both the magnitude of the serum GtH concentration as well as the rate of increase in circulating GtH levels, as a large but slow increase in serum GtH levels, such as by injection of a high dosage of LHRH-A alone (Chang and Peter 1983b; Sokolowska et al. 1984, 1985a, 1985b) or by implants of pellets containing a large dosage of LHRH-agonist (Sokolowska et al. 1984), were not effective in inducing ovulation in goldfish. Thus, in the normal *in vivo* situation, removal of the dopamine inhibition and an increase in GnRH stimulation of GtH secretion are probably required for the rapid increase in circulating GtH levels for ovulation.

Studies on a number of other teleosts indicate that injection of LHRH-A or other agonist analogs of LHRH are effective in stimulating GtH secretion although, with the exception of salmonids, injection of LHRH-A alone is relatively ineffective in stimulating ovulation. Studies performed to investigate the role of neurotransmitters or catecholaminergic drugs in the neuroendocrine regulation of GtH release in several teleosts indicate that dopamine acts as a GRIF on spontaneous and/or GnRH-stimulated GtH release.

Lin et al. (1985b, 1985c) reported that in the Chinese loach, *Paramisgurnus dabryanus*, injection of the dopamine receptor antagonist pimozide or drugs that block the synthesis of dopamine, such as reserpine, alpha-methyl-para-tyrosine and carbidopa, potentiate the effects of LHRH-A on GtH secretion, and that the combination of any of these drugs with LHRH-A is highly effective in inducing ovulation in this species. However, injection of diethyl-dithio-carbamate, which blocks the conversion of dopamine to norepinephrine, had no effects on potentiating the actions of LHRH-A. In common carp *(Cyprinus carpio)* (Billard et al. 1983; Lin et al. 1985c), bream *(Parabramis pekinensis)* (Lin et al. 1985c), and mud carp *(Cirrhinus molitorella)* (Lin et al. 1985d) injection of pimozide potentiated the ability of LHRH-A to stimulate GtH release and induce ovulation. Lin et al. (1985c, 1985d) also reported that reserpine treatment activated the ability of LHRH-A to cause increased circulating levels of GtH and induce ovulation in the common carp and mud carp. These results demonstrate that in the Chinese loach and the common carp, blockage of dopamine actions by inhibiting synthesis or blockage of dopamine receptors remove its inhibitory influence on GtH release, enhancing the GtH release-response to LHRH-A. Preliminary studies also indicate that the combination of treatment with pimozide or reserpine and LHRH-A is effective in inducing ovulation in grass carp *(Ctenopharyngodon idella)*, silver carp *(Hypophthalmichthys molitrix)* and bighead carp *(Aristichthys nobilis)* (Lin et al. 1985d).

De Leeuw et al. (1984) reported that spontaneous release of GtH from cultured pituitaries of the African catfish *(Clarias lazera)* could be inhibited by apomorphine. Furthermore, injection of pimozide was found to increase plasma GtH levels in juvenile catfish and to activate the ability of LHRH-A to stimulate GtH release in both juvenile and mature catfish. The combination of pimozide and LHRH-A was also effective in inducing ovulation (de Leeuw et al. 1985a, 1985b). Results from studies on the effects of pimozide on the response to injection of LHRH agonists in the walleye, *Stizostedion vitreum*, are consistent with the existence of an inhibitory influence of dopamine on GtH release (Pankhurst et al. 1985).

In salmonids, it is well established that injection of LHRH superactive analogs alone stimulates a marked increase in blood levels of GtH and ovulation (Sower et al. 1982; Donaldson et al. 1983; Crim et al. 1983; Van Der Kraak et al. 1985, 1986). However, in spite of this sensitivity to LHRH and superactive analogs greater than in other teleosts, an inhibitory dopamine influence on spontaneous as well as GnRH-stimulated GtH release is also present. Billard et al. (1985) reported that pimozide increased the GtH release-response to injections of an LHRH agonist in rainbow trout *(Salmo gairdneri)* and brown trout *(Salmo trutta)*. In rainbow trout, injection of apomorphine was reported to inhibit the postovulatory GtH release (Gielen, unpublished results, cited by de Leeuw et al. 1985b), and dopamine was found to inhibit the release of GtH from pituitaries incubated *in vitro* (Crim 1981). In the coho salmon *(Oncorhynchus kisutch)*, pimozide injection also increased the basal as well as the LHRH-A-stimulated GtH secretion *in vivo* (Van Der Kraak et al. 1986).

Refinement of Induction Methods

It is clear that the GRIF action of dopamine is found in a wide phylogenetic range, and perhaps in all teleosts (Peter et al. 1986). In many teleosts the endogenous GRIF activity of dopamine can effectively modulate the GtH-releasing activity of exogenous GnRH to the extent that a high rate of ovulation cannot be induced by injection of superactive GnRH analogs. The use of catecholaminergic drugs that block the action or synthesis of dopamine in combination with LHRH-A, or other superactive analogs of GnRH (Peter et al. 1985), represents an effective means of inducing ovulation in a number of teleosts. Research in the future can refine this new technique of inducing ovulation by determining the optimal dosages of drugs appropriate for specific species.

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Collection of Naturally-Spawned Milkfish Eggs in Floating Cages

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Abstract

Natural spawnings of milkfish from floating cages were obtained from different stocks of 5-7 year-old milkfish in 1980, 1981, 1983 and 1985. The maximum number of eggs collected in 1980, using a series of 1-m diameter stationary plankton nets, was about 900. Increased collections were obtained in succeeding years with different types of egg collectors and methods. Egg collection, however, is inferior when based on the expected number of eggs spawned by a single female. Although no systematic study was attempted to compare efficiency of various egg-collecting gears and methods, the problems associated with the use of each gear are presented. The experience may be used as a guide in future designs of efficient gears for collecting naturally-spawned eggs of milkfish or other fish species in floating cages.

Introduction

Milkfish *(Chanos chanos* Forskal*)*, an important food fish in the Philippines and Southeast Asia spawns naturally in floating cages (Marte and Lacanilao 1986), earthen ponds (Lin 1984, 1985) and tanks (Poernomo et al., unpublished data). Spontaneous spawning following administration of gonadotropin hormone releasing hormone-analogue (GnRH-A) was also recently reported (Lee et al. 1986; Marte et al., in press).

The maximum number of spawned eggs collected by Lin (1985) after one spawning of milkfish breeders held in earthen ponds was 7.2 million. Egg collection from milkfish held in floating cages is by comparison inferior. Inefficient egg collection from floating cages is an important problem which requires an early solution if mass production of milkfish fry is to be realized.

This paper presents observations on milkfish spawning, the various types of egg collectors used and the methods of egg collection from floating cages. The problems inherent in egg collection from floating cages and those associated with the use of each type of collector are discussed.

Materials and Methods

Milkfish broodstock were held in 9 x 10 x 3 m deep cages located in a protected cove at SEAFDEC's Igang substation, Guimaras Island, Philippines. Water depth at the cage site was 7 m and water transparency was 3-6.5 m. Current velocity at slack tide was a round 1 cm/sec. The cage number, cage dimension, age and source of broodstock, number of fish per cage, and type of egg collector used are shown in Table 1.

The egg collectors tested were:

1. Type A stationary plankton net (Fig. 1) 1 x 2 m plankton net (1 mm mesh). Six nets were set outside the cages at three levels (surface, middle, and bottom) of the net cage on opposite sides of the cage along the direction of the current.
2. Type B stationary plankton net (Fig. 2) an elliptical frame, 2 x 1 m constructed of PVC pipe lined with plastic sheet. A detachable plankton net (1 m long, 1 mm mesh) was attached to the frame. Two frames were set on each side of the net cage below the water surface.
3. Type C stationary plankton net (Fig. 3) similar in construction to the Type B net. A series of five frames were set 1 m away from the net cage on opposite sides and along the current direction.
4. Egg sweeper (Fig. 4) constructed of two parallel bamboo poles connected to a central shaft and supported by bamboo frames. Attached to the lower pole was a seine net, 5 x 1 m provided with a detachable conical bag at one end. The upper pole served as a handle and guide for the lower pole. The sweeper was operated manually by turning the upper pole in a counterclockwise direction around the cage. The cage was lined with plastic-coated sack from the water surface to 1 m above the cage bottom.
5. Seine net (Fig. 5), a fine 'jersey' cloth (0.80 mm mesh) 10 m x 1 m with a conical bag at the center and supported at both ends by bamboo poles. Rubber floats

and lead sinkers were attached to the float and sinker lines, respectively. Collection was done by two persons swimming around the cage moving towards one side while pulling the seine closer to concentrate the eggs. The cage was lined with plastic-coated sack.

Plankton nets were inspected daily between 0600 and 0800 hr. The egg sweeper was operated every hour between 0200 and 0600 hr whenever spawning behavior or increased swimming activity was observed. Seining was done from 0400 to 0600 hr whenever eggs were found in seawater sampled from the cage.

Results

The number of spawnings of milkfish broodstock varied from two in 1980 to 35 in 1985 (Table 1). The least efficient gear for collecting spawned eggs was the Type A stationary plankton net. Improved collections were obtained with the larger Type B net used in 1983. Increasing the number of nets, however, did not increase collection. Using the seine net and egg sweeper gave slight improvements in egg collection.

In 1985, milkfish spawnings from captive stock occurred from April to October (Table 2). The peak spawning was in June-July with a total of 24 spawnings. Spawnings decreased in August and were few in September and October.

Spawning activity seems to be related to the moon phase. More spawnings were observed throughout the first quarter and the full moon. The maximum number of eggs collected during one spawning was also greater during these periods (Table 3).

When spawnings occurred, increased swimming activity was observed from late afternoon to early evening. Activity in the cage became more pronounced from 2000 hr. Chasing and occasional leaping were observed while the sound of water slapping could be heard even from a distance. Younger broodstock in the other cages were quiescent during this time.

Discussion

Egg collection was improved with the use of manually-operated seine net and egg sweeper. Lining the net cage with plastic coated sack was effective in retaining spawned eggs. The cage lining did not seem to affect the fish and water exchange through the unlined cage bottom was adequate.

The number of eggs collected from the floating cage is inferior compared to those collected by Lin (1985) from his ponds. Collection is at best about 75% of the expected number spawned by a single female, and most were from 5-20% of the expected number which can be spawned by a female. Fecundity of captive broodstock is about 300,000/kg (Marte and Lacanilao 1986). Spawnings were more frequent during the first quarter to full moon phase. These observations, however, do not fully agree with Kumagai (1981) who reported more frequent egg collection from plankton tows during the first and last quarter moon periods.

A major problem unique in egg collection from floating cages is the presence of numerous egg predators such as cardinal fish and anchovy inside the cages. These were observed to increase during the breeding season. They were often caught while gathering the eggs, and their stomach contents contained hundreds of milkfish eggs. Carnivorous fish such as sea bass could be stocked together with milkfish to minimize the number of egg predators. The effect on the milkfish breeders of another species in the cage and the appropriate size and number of another species to be stocked still have to be evaluated.

The stationary plankton nets are easy to install and use but are very inefficient because collection is dependent on the current velocity. In addition, the plastic lining on the larger nets become heavily clogged with algae and floating debris. The egg sweeper is relatively convenient as it can be operated by one person from the cage platform. However, it is bulky and difficult to install. The permanent presence of the lower pole with the overhanging net seem to inhibit spawning. There were few spawnings observed from this cage (Cage 42) although the number of fish stocked was twice that in Cage 43 where collection was done by manual seining. Fish obtained from this cage (Cage 42), after a prolonged period when no spawning was observed, were spermiating males and mature females. Manual seining, although relatively more efficient, is laborious and requires at least one person swimming inside the cage long before dawn.

Methods to improve egg collection from floating cages are currently being tested. This includes structural improvements on the egg sweeper, the use of carnivorous fish to reduce the number of egg predators, and the adoption of a paddlewheel aerator to direct water current towards a collecting net. Alternatively, handling methods such as the transfer of spawners to spawning tanks before the breeding season or just prior to the onset of spawning will be tried.

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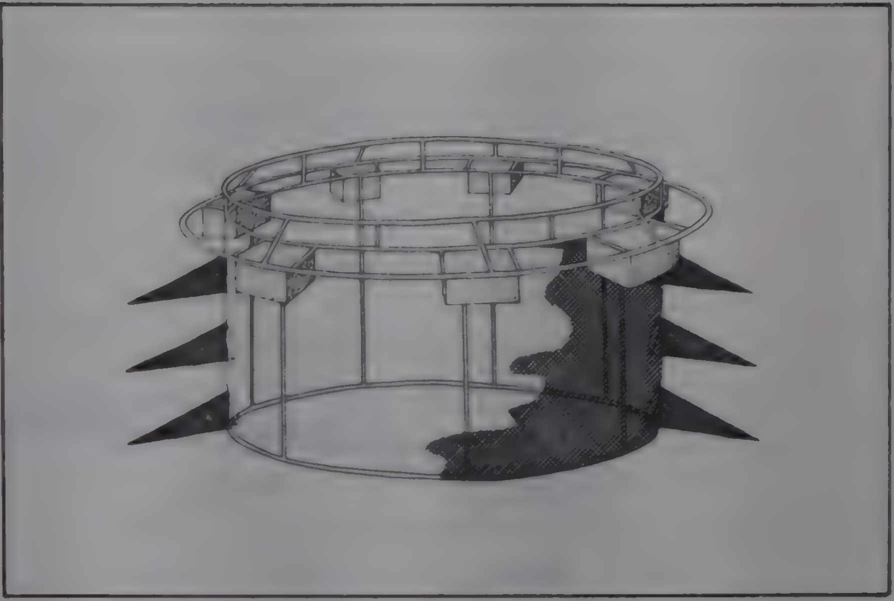


Fig. 1. Position of plankton nets used as egg collectors beside the maturation cage, Type A net.

Table 1. Egg collector, spawning frequency and egg collection, 1980-1985.

Egg collector	Year	Cage	Number of spawnings	Number of eggs collected
Type A stationary plankton net	1980	C-5	2	500– 900
Type A stationary plankton net	1981	C-6	8	342– 6,293
Type B stationary plankton net	1983	C-2	14	770–114,000
Type C stationary plankton net	1985	C-5	1	1,050
Seine net	1985	C-43	35	1,500–769,000
Egg sweeper	1985	C-42	6	4,280–212,000

Table 2. Spawning frequency of milkfish broodstock and egg collection, 1985.

Month	Spawning frequency (No./mo.)	Eggs collected (No./mo.)
April	2	1,050 – 2,900
May	3	6,300 – 212,500
June	10	4,200 – 463,000
July	14	1,500 – 259,000
August	6	36,000 – 636,500
September	3	80,900 – 769,500
October	3	30,700 – 110,400

Table 3. Spawning frequency of milkfish broodstock and egg collection in relation to moon phase, 1985.

Moon phase	Number of spawning	Number of eggs collected
New Moon	5	2,900 – 335,000
First Quarter	12	1,050 – 769,500
Full Moon	16	6,900 – 636,500
Last Quarter	8	1,500 – 145,200

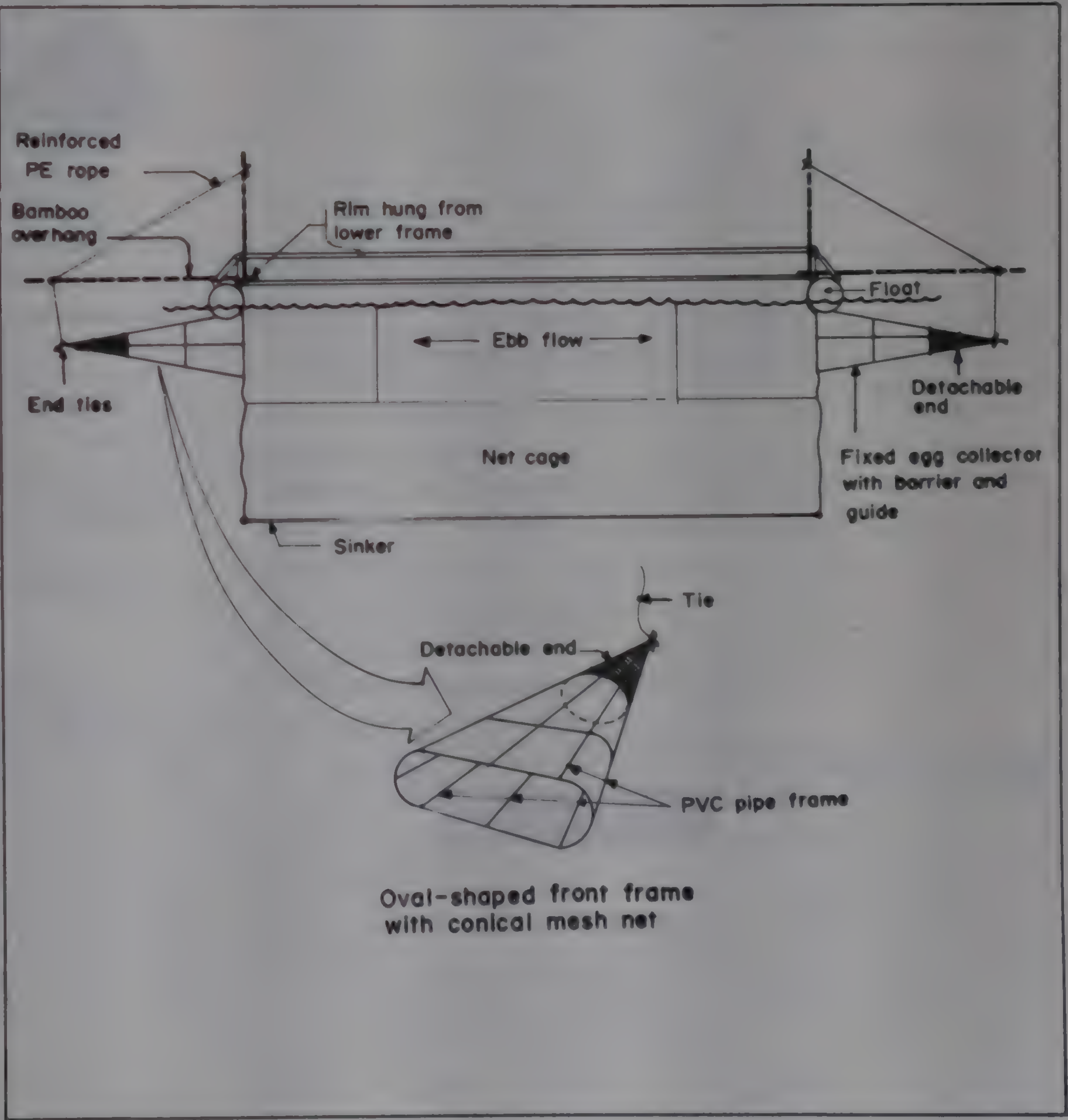
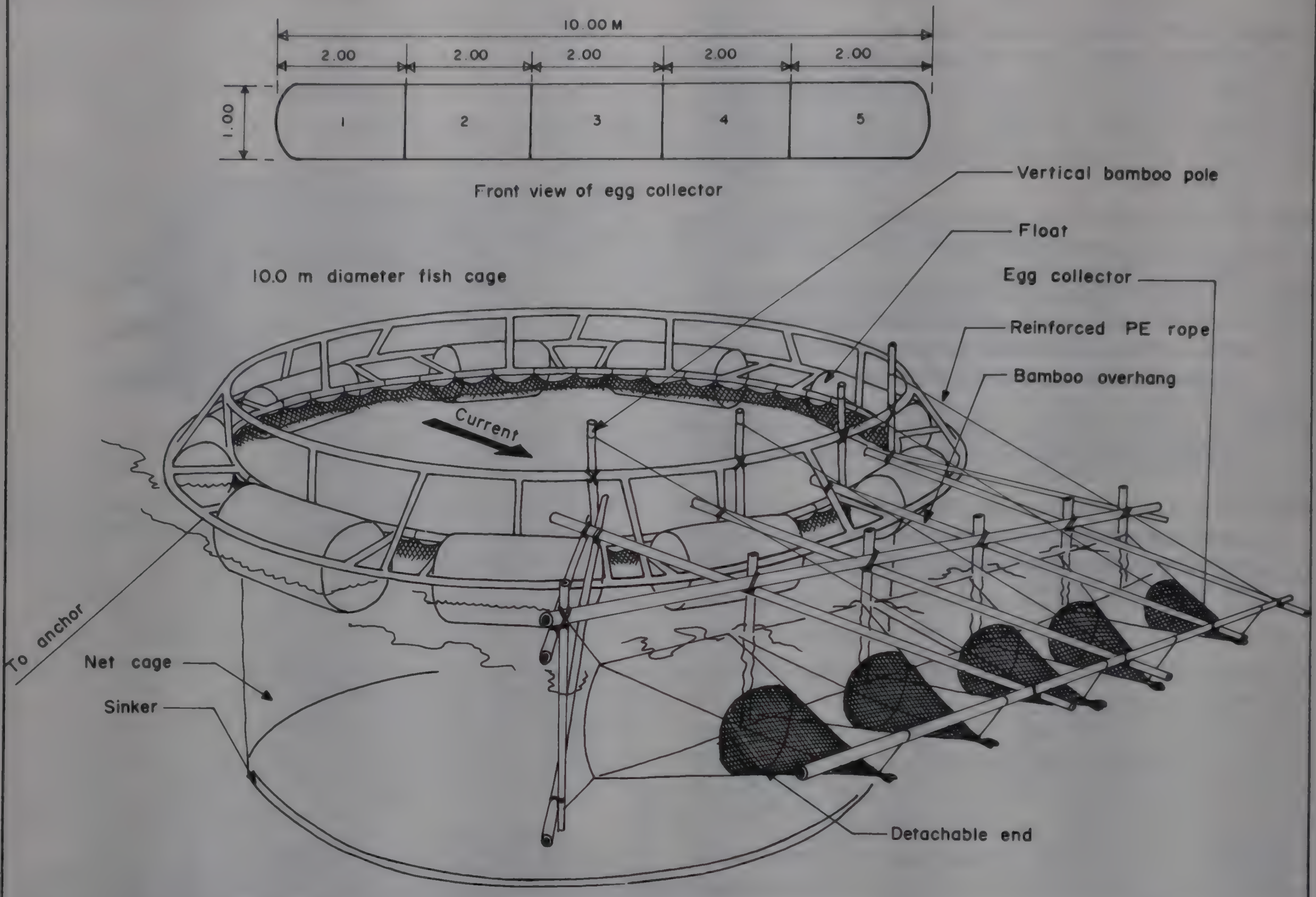


Fig. 2. A combination of egg barrier net and egg collector set at opposite side, Type B net.


10.00 M
2.00
2.00
2.00
2.00
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1.00
1
2
3
4
5
Front view of egg collector
Vertical bamboo pole
Float
10.0 m diameter fish cage
Egg collector
Reinforced PE rope
Bamboo overhang
Current
To anchor
Net cage
Sinker
Detachable end

Fig. 3. Series of five plankton nets set on opposite sides of the maturation cage.

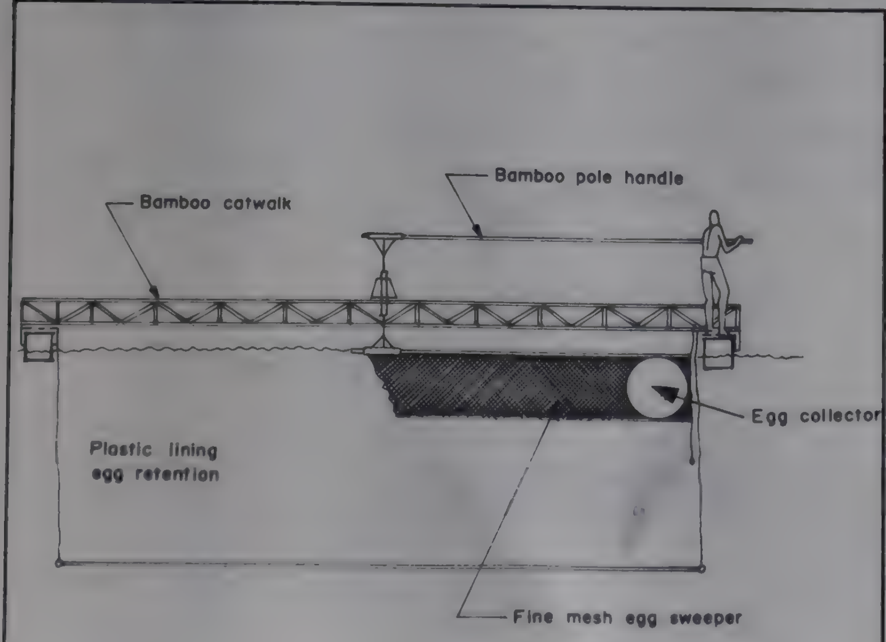
Bamboo pole handle
Bamboo catwalk
Egg collector
Plastic lining egg retention
Fine mesh egg sweeper

Fig. 4. "Egg sweeper".

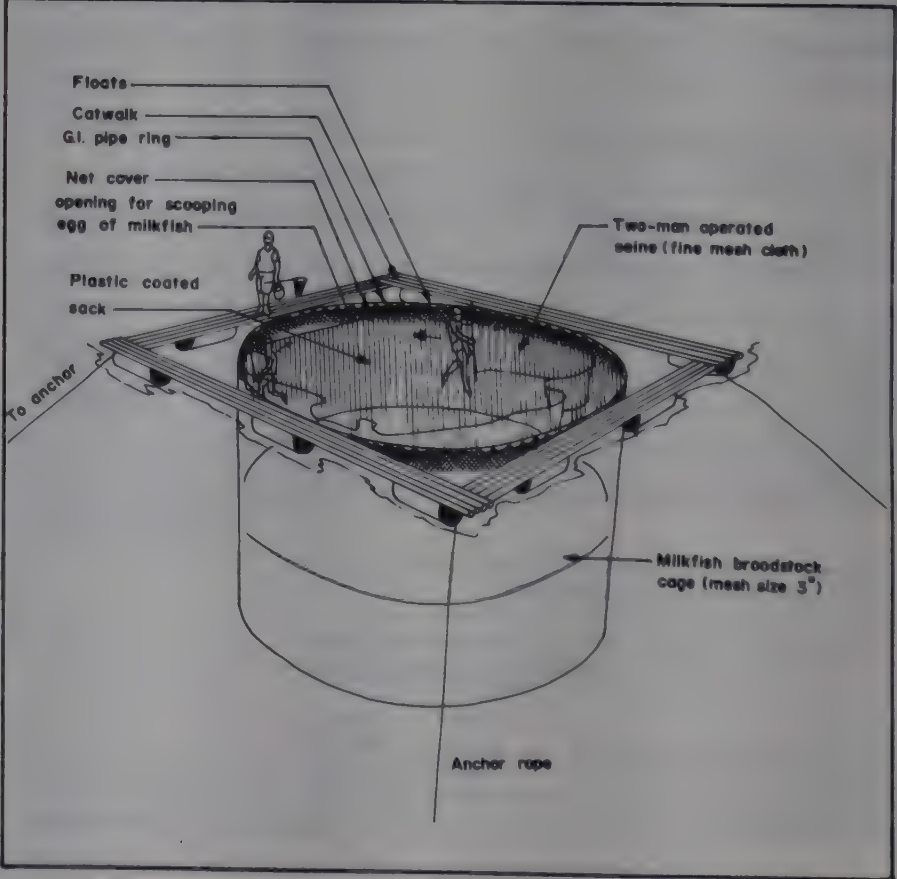
Floats
Catwalk
G.I. pipe ring
Net cover
opening for scooping egg of milkfish
Plastic coated sack
Two-man operated seine (fine mesh cloth)
To anchor
Milkfish broodstock cage (mesh size 3")
Anchor rope

Fig. 5. Egg collection by manual seining.

Evaluation of a New Androgen (Mibolerone) and Procedure to Induce Functional Sex Reversal in Tilapia

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Abstract

Sexually undifferentiated tilapia *(Oreochromis aureus)* fry (9-11 mm total length) were exposed in 38-l aquaria for four and six weeks at 0 (control), 0.1, 1.0 and 10.0 ppm of ethynyl testosterone (ET) and mibolerone (MI) and then grown to 60 mm. Gonadal sex was determined using gonadal squash analyses on 50 fish per treatment replication. A concentration of 10.0 ppm MI for 24 hours was lethal to tilapia fry. Sex reversal to a male state was accomplished in four treatments: 1.0 ppm MI (four and six weeks), 0.1 ppm MI (six weeks) and 10.0 ppm ET (six weeks). Exposure to 1.0 ppm MI for four and six weeks resulted in an average of 62.3% males and 37.7% ovotesticular fish (intersex or sterile); no females were produced. All other androgen treatments yielded gonadal females. The ovotestes of the 1.0 ppm MI-treated fish consisted of degenerate gonad strands containing very few primary oocytes. These ovotesticular fish would probably be functionally sterile. Ovotestes in fish from all other androgen treatments contained a comparatively greater number of primary oocytes, which might function as ovaries with reduced reproductive capacity.

Introduction

All-male populations of tilapia are often preferred over mixed or all-female populations because of the faster growth rate exhibited by males starting at a size of 100-150 g. Stocking only male tilapia in ponds usually results in larger fish and thus a more valuable crop. Monosex tilapia culture, by eliminating unwanted reproduction, allows the fish farmer to grow and harvest fish at a selected size, and removes the need to totally drain ponds to avoid stunting problems.

All-male populations of tilapia have been produced by orally administering androgen hormones to fish beginning in the early fry stage (Guerrero 1975). Functional sex reversal is achieved by administering the androgen to sexually undifferentiated fry (less that 12 mm length) over a three to four week period. The most popular method incorporates an androgen such as methyl or ethynyl testosterone into the feed (Hepher and Pruginin 1981). With this method, it has been suggested (Yamamoto 1958) that natural foods be available to the fry only at minimal levels, as consumption of the androgen-treated feed may be reduced in proportion to the amount of natural food consumed. However, tilapia sex reversal has been achieved in the presence of natural foods (Chambers 1984). Another possible problem with the oral administration method is that at high fry densities in treatment tanks, feeding aggression and competition may result in differential consumption of the androgen-treated feed, which might result in instances of incomplete sex reversal. Multiple daily feedings partially compensate for this behavior. However, this difference in feeding aggression is intensified when fry of even slightly different sizes are treated together.

Sex reversal of tilapia (Yashouv and Eckstein 1965; Hackman 1974) and other cichlids (Nakamura 1975) has been achieved to a limited degree by dissolving hormones in the water containing fish fry or eggs. This method permits initiation of sex reversal treatment prior to the feeding stage of fry (Goetz et al. 1979), and allows sex reversal applications to a wider variety of fish species. Use of the immersion method could therefore eliminate problems inherent in the oral administration method. However, the literature cited indicates that induction of sex reversal by the immersion method may require increased amounts of androgen as compared to the oral administration method. Therefore, the relative potency of the androgen for use in immersion treatments is a potentially important selection criterion, to reduce both direct costs and toxic drug levels.

Mibolerone is a synthetic anabolic steroid, related to testosterone (Zimbelman 1978) (Fig. 1), which has androgenic activity (Lyster and Duncan 1963). Mibolerone (registered as Cheque by the Upjohn Company, Agricultural Division, Kalamazoo, Michigan 49001, USA) has been shown to be highly effective in preventing estrus in dogs by blocking the luteinizing hormone (LH) surge. LH is a gonadotropic hormone produced by the teleost pituitary gland which is important in gonadal development. When compared to methyl testosterone, an androgen commonly administered orally to effect sex reversal in tilapia, mibolerone was 16 times more potent as an androgen in rats (Lyster and Duncan 1963). However, no previous research has been performed with mibolerone to induce sex reversal in fish.

The purpose of this study on *Oreochromis aureus* was to compare phenotypic sexual development using various levels of mibolerone and ethynyl testosterone in solution during the period of sexual differentiation.

Materials and Methods

The sex reversal study was conducted in static water aquaria at approximately 27 ±2°C. Each 38-l aquarium was aerated and stocked with 250 9-11 mm fry randomly collected from an outdoor spawning pond. The fish were fed approximately 10% of their body weight daily during the first three weeks of treatment, with a gradual reduction to 6% in the remaining three weeks. All fish were fed a 42% protein trout diet.

Mibolerone and ethynyl testosterone (ET) were the androgens used in the sex reversal immersion treatments. Each androgen was suspended at 0 (control), 0.1, 1.0 and 10.0 ppm, using three replications per trial (Table 1). Dimethyl sulfoxide (2.6 ppt) was used to promote exposure of the dissolved androgens to the fish, due to the relatively low solubility of mibolerone in water. No dimethyl sulfoxide was used in the control treatment water. Five fish from each aquarium were weighed and measured each week to establish weekly feeding rates. Aquaria were drained weekly and refilled with dechlorinated and filtered tap water, at which time the test androgens were replaced at the appropriated dosage. Aquaria were siphoned twice weekly to remove uneaten feed. A 14:10 hour light:dark cycle was maintained.

Fish were exposed to the androgens for four or six weeks. Survival was based on six weeks of androgen exposure. Fish were then grown out to at least 60 mm (total length) in flow-through tanks for gonad analysis using the aceto-carmine squash technique (Guerrero and Shelton 1974). Fifty fish per aquarium (150 fish per treatment) were sacrificed for gonadal analysis. Fish with gonads containing no oocytes were classified as males, while gonads containing both oocytes and testicular tissue were classified as ovotestes. Ovotestes are gonads containing both ovarian and testicular tissue, and manifest themselves in squash analyses by the presence of primary oocytes embedded in testicular tissue (Meriwether and Shelton 1980). The binomial frequency distribution was used to calculate statistical deviations from the normal female:male gonadal sex ratio of 50%. Analysis of variance and Duncan's Multiple Range Test were used to compare final weights of fish after six weeks of treatment.

Results

Exposure of tilapia fry to a 10.0 ppm mibolerone solution resulted in 100% mortality within 24 hours. Fish survival during the six-week treatment period ranged from 66.1% to 85.1% in the remaining six treatments (Table 1).

Average fish growth after six weeks of treatment was highest in the control treatment and lowest in the 1.0 ppm mibolerone and 10.0 ppm ET treatments (Fig.2). Increasing concentrations of each androgen appeared to have a negative effect on fish growth; the final average weight of control (untreated) fish was significantly higher than that of fish treated with 10.0 ppm ET, 1.0 ppm ET, and 1.0 ppm mibolerone.

Excluding the 10.0 ppm mibolerone treatment, immersion exposure to the androgens resulted in a significant increase in the percentage of gonadal males in four treatments; 1.0 ppm mibolerone for four weeks, and 0.1 ppm mibolerone, 1.0 ppm mibolerone, and 10.0 ppm ET for six weeks (Table 1). All androgen treatments contained fish with ovotesticular gonads. Exposure to 1.0 ppm mibolerone for four and six weeks resulted in 63.3% and 61.3% males, respectively, with the remainder being ovotesticular fish; no gonadal females were produced in either treatment. All other androgen treatments contained some gonadal females (range = 0.7% to 49.3%).

Discussion

Survival of tilapia exposed to the androgens ranged from 66.1% to 80%, which is poor compared to typical sex reversal treatments utilizing the oral administration method over similar time periods (Hopkins et al. 1979). The relatively low survival rates (except for the 10.0 ppm mibolerone treatment) were partially attributed to the stagnant water in the static-water aquaria. Survival may be increased by the use of antibiotics in the feed, more frequent water exchange or by greater aeration than that used in this study. Use of larger tanks during immersion treatments would also permit daily siphoning without frequent water replacement.

Continuing the androgen exposure for six weeks resulted in an average 7.6% gonadal males produced across all treatments (from Table 1). As some fry were only 16 mm in total length after four weeks of treatment, it is likely that the growth-inhibiting effect of the androgens (Fig. 2) extended the time required for some of the tilapia fry to complete sexual differentiation.

Functional sex reversal was achieved using the immersion method, since a significantly higher ($P = 0.05$) percentage of gonadal males was produced in four treatments. Mibolerone was much more effective than ET in achieving sex reversal, requiring only about 1% of the ET concentration to achieve similar results.

The ovotestes of the 1.0 ppm mibolerone-treated fish consisted of degenerate gonad strands containing very few (usually less than five) primary oocytes. The ovotesticular fish would probably be functionally sterile as females and incapable of reproduction due to physiological and behavioral constraints (Meriwether and Shelton 1980). Ovotestes in fish from all other androgen treatments contained a comparatively greater number of primary oocytes, which, depending on the relative abundance of oocytes, might function as ovaries with a reduced reproductive capacity.

Exposing undifferentiated tilapia fry to aqueous solutions of mibolerone appears to be a feasible method for eliminating production of gonadal females. Based on preliminary study results, exposure to 1.0 ppm mibolerone for four to six weeks appears to be an effective treatment, resulting in treated fish that should be incapable of reproduction as females. Use of the immersion method, with a potent androgen such as mibolerone, may be an effective method to induce sex reversal in fishes demonstrating early development of sexual differentiation. Further studies are being conducted to further define effective treatment rates and conditions to induce functional sex reversal in tilapia exposed to mibolerone in solution.

NOTE: Mibolerone is currently available for nonresearch applications by veterinary prescription only, registered as Cheque, consisting of mibolerone dissolved in propylene glycol. Relative cost comparisons of mibolerone with other synthetic androgens, for use on commercial-scale applications, are therefore not available; however, mibolerone production costs by the manufacturer are considered to be low (Zimbelman 1978).

Acknowledgements

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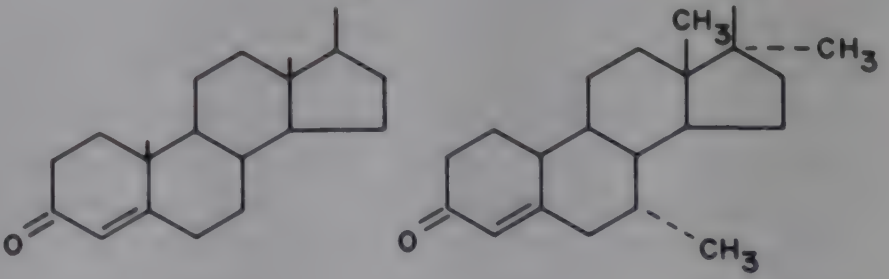
Table 1. Gonadal sex and survival of *Oreochromis aureus* fry exposed to various concentrations of mibolerone (MI) and ethynyl testosterone (ET) in solution.

Treatment (ppm)	4-week exposure			6-week exposure			
	% Male	% Female	% Ovotestes	% Male	% Female	% Ovotestes	Survival (%)[a]
0.1 ET	38.0	49.3	12.7	46.7	38.7	14.7	70.0
1.0 ET	42.0	7.3	50.7	66.7	3.3	30.0	68.1
10.0 ET	52.0	9.3	38.7	60.6[b]	0.7	38.7	80.0
0.1 MI	56.7	3.3	40.0	62.0[b]	0.7	37.3	78.3
1.0 MI	63.3[b]	0.0	36.7	61.3[b]	0.0	38.7	73.1
10.0 MI	–	–	–	–	–	–	0
0 (Control)	46.0	54.0	0.0	52.0	48.0	0.0	85.1

[a] Survival calculated for six-week exposure treatments

[b] Significantly different from 50.0% at the P = 0.05 level



Testosterone

Mibolerone

Fig. 1. Chemical structures of testosterone and mibolerone (from Zimbelman 1978).

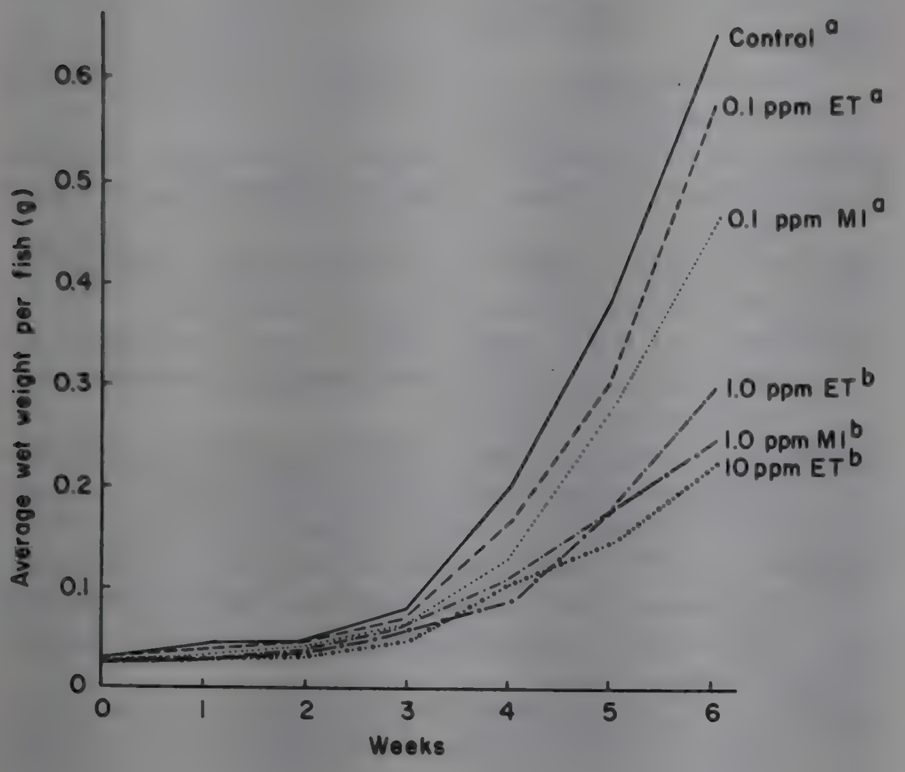


Fig. 1. Growth of *Oreochromis aureus* fry during immersion exposure to ethynyl testosterone (ET) and mibolerone (MI) of various concentrations. Final average weights with different superscript letters are significantly different at a $P = 0.05$ level.

Variations in Egg Qualities of the Japanese Whiting, *Sillago japonica,* in the Spawning Season

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OOZEKI, Y. and R. HIRANO. 1986. Variations in egg qualities of the Japanese whiting, *Sillago japonica*, in the spawning season, p. 679-682. *In* J.L. Maclean, L.B. Dizon and L.V. Hosillos (eds.) The First Asian Fisheris Forum. Asian Fisheries Society, Manila, Philippines.

Abstract

Changes in the egg quality of the Japanese whiting, a multiple-spawning fish, were examined throughout its spawning season. Fertilization and hatching rates were constantly high and the optimum temperature and salinity for hatching (26.0°C, 32.5 ppt) did not vary much. Oxygen consumption was constant at each developmental stage and linearly increased as egg development progressed. Eggs were damaged by exposure to temperatures higher than 28°C for 15 min. The thermal tolerance of eggs varied at each development stage. High sensitivity to temperature shock was observed at two periods, from fertilization to blastula and from middle gastrula to formation of optic vesicles. During the latter period, TL_{50}, defined as the minimum shock temperature after which 50% eggs hatched normally, was observed to be higher in the peak spawning period than in the final spawning period. The decrease of TL_{50} in the final spawning period agreed well with the survival activity of starved larvae which decreased in the same period.

Introduction

To determine the early life history of marine fish and to develop seed production techniques, it is necessary to know the tolerance of eggs and larvae to changing environmental conditions. It has long been recognized that the egg stage is more sensitive to stress than the other development stages. Knowledge of the variations in the egg quality of multiple-spawning fish during their long spawning season is necessary because these fishes are very important for seed production. However, little information on the changes of egg quality during their spawning is available. Mito et al. (1969) described the changes of fertilization and hatching rates during one spawning season of the stone flounder and Lee (1981) reported the relationship of egg diameter and spawning conditions during the spawning season of Japanese whiting. This report describes changes in the egg condition of the Japanese whiting, *Sillago japonica* Temminck et Schlegel with respect to optimum condition for hatching, respiration, thermal tolerance of eggs and larval survivability, between the peak and final spawning period (Mito et al. 1969).

Materials and Methods

Spawners of Japanese whiting were collected by angling in Lake Hamana, a saline lake on the Pacific coast of Honshu, Japan. Several groups of one female and two or three males were selected and placed in spawning tanks, after hormone injection (synahorine 50 R.U./dose, dissolved in 0.2 ml physiological salt solution). Seawater from Lake Hamana was supplied to the tanks at daytime. Water supply was stopped at night in order to collect spawned eggs. No overmatured eggs were found. The spawning season was divided into three periods, after Mito et al. (1969) and the division of developmental stages followed Oozeki and Hirano (1985).

Oxygen consumption was measured with a differential gas volumeter (Yokohama 1969). These values were measured for about 200-1,000 eggs every two hours from the 16-cell stage to hatching at 26.0°C.

Batches of 200 eggs put into 48 one-liter beakers were incubated under 48 different combinations of salinity and temperature. The salinity ranged from 10.5 to 49.0 ppt at intervals of 5.5 ppt and the temperature ranged from 20.0 to 30.0°C at intervals of 2.0°C. After hatching, normal, abnormal and dead larvae and dead eggs were counted. The percentage of eggs that hatched normally was calculated by the following formula:

(normally hatched larvae)/(total eggs) x 100%.

Batches of 500 eggs were put into 22 one-liter beakers with 0.9 l seawater and incubated at 24.0°C. The development of the eggs was examined every hour. Simultaneously, several groups of 50 eggs were transferred to 100-ml beakers containing 80 ml seawater set at the shock temperatures of 24.0, 32.0, 34.0, 36.0, 38.0, 40.0 and 42.0°C. After 15 min. of exposure at the shock temperature, each 100-ml beaker was placed back in the water bath and maintained at the initial temperature of 24.0°C until the end of the examination. After hatching, larvae and dead eggs were counted. The percentage of eggs that hatched normally was calculated by the above formula.

To determine larval survivability, two groups of 100 eggs were each put into a 1-l beaker containing 0.9 l of seawater at 24.0°C. Newly-hatched larvae were reared without feeding and their survival rates were calculated every 12 hours.

Results and Discussion

The percentage of eggs that hatched normally at the natural spawning temperature was more than 95%, except in one experiment when the spawning temperature was very high at 29.1°C.

Oxygen consumption was constant at each development stage, increasing as egg development progressed, from 6.20 x 10^{-3} µl O_2/egg/hr (0.70 µl O_2/mg dry wt/hr) at the 32-cell stage to 53.1 x 10^{-3} µl O_2/egg/hr (6.02 µl O_2/mg dry wt/hr) just before hatching (Fig. 1). These findings are almost the same as those of Lasker and Theilacker (1962) for sardine eggs. Throughout the spawning season, no changes in oxygen consumption at each development stage were found.

In Fig. 2, five experiments were compared to evaluate the changes of hatching activity of eggs during the spawning season. The relation between normal hatching rate (Y) and temperature-salinity condition (T, S) was calculated by the orthogonal polynominal method and expressed by the equation:

$$Y = a_0 + a_1 T + a_2 S + a_{11} T^2 + a_{22} S^2 + a_{12} TS$$

(a: constant)

Table 1 shows the spawning season and survival activity index of starved larvae (SAI). SAI is expressed by the equation (Shimma and Tsujigado 1981):

$$SAI = \frac{\sum_{i=1}^{k} S_i \times i}{N}$$

where N is the total number of larvae, S_i the number of survived larvae at *i*-th day and k the number of days until all larvae die due to starvation. Positive correlations were observed between the spawning temperature-salinity and the calculated optimum normal hatching conditions. The ranges in temperature and salinity, resulting in more than 90% normal hatching, varied with changes in spawning conditions (Fig. 2). No large difference in the ranges resulting in 90% normal hatching was observed between the peak spawning period and the final one. Moreover, no relation was found between the ranges resulting in 90% normal hatching and SAI (Table 1). In one exception, eggs spawned on 17 August showed a low normal hatching rate, which could have resulted from the high spawning temperature (29.1°C).

Oozeki and Hirano (1985) described two short phases of egg development very susceptible to high temperature shock. These were "from fertilization to blastula" and "from middle gastrula to optic vesicles formation". Oozeki and Hirano (1985) used the minimum shock temperature, between the middle gastrula stage and the optic vesicles formation stage, which resulted in 50% of eggs hatching normally, as an indicator of thermal tolerance expressed as TL_{50}. Table 2 shows SAI from 5.60 to 8.45 and TL_{50} from 34.4 to 36.5°C for three different spawners. Table 3 shows the daily changes of SAI and TL_{50} of eggs from one spawner during a week, with SAI from 6.00 to 8.47 and TL_{50} from 34.2 to 35.5°C. It follows that the differences of TL_{50} among spawners are equal to the daily variations for one spawner during the peak spawning period and that there may be no relation between the values of SAI and spawning conditions. However, Table 4, a comparison of SAI and TL_{50} for the same spawners for two different spawning periods, shows that both values of the final period were lower than those of the peak spawning period.

In Japanese seed production centers, it is said that the survival of hatched larvae is lowered along with the decrease in fertilization and hatching rates at the end of the spawning season. We confirmed that the high temperature tolerance of eggs and the survival of larvae clearly dropped during the final spawning period even for normal hatching rates of more than 95% in our experiments. In contrast, oxygen consumption and optimum temperature-salinity conditions for normal hatching did not vary between the second spawning period and the final one. These findings suggest that deterioration of egg quality, indicating larval survival is due not only to death or the cessation of development processes but also to a decrease in environmental tolerance during the egg stage.

Acknowledgements

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Table 1. Survival activity index (SAI) of eggs spawned during different spawning seasons. SAI was calculated using the relation

$$SAI = \left(\sum_{i=1}^{k} (S_i \times i) \right) / N$$

where N is the total number of larvae, S_i is the number of survived larvae at i-th day, and k is the number of days until all larvae die due to starvation. Each number corresponds to that in Fig. 2.

No.	Date	SAI	Spawning period
1	7.19	6.97	second period
2	8.17	6.99	second period
3	8.26	7.97	second period
4	9.15	6.39	final period
5	9.19	4.05	final period

Table 2. SAI and TL_{50} of eggs from three different spawners. The values of SAI were obtained as described in Table 1. TL_{50} was the minimum shock temperature which resulted in 50% of eggs hatching normally.

Spawner	SAI	TL_{50}
A	8.45	34.4 °C
B	5.98	36.5
C	5.60	34.9

Table 3. Daily changes of SAI and TL_{50} of eggs spawned from one spawner. The values of SAI was obtained in the same manner as described in Table 1.

Date	SAI	TL_{50}
7.21	6.57	34.2 °C
22	6.00	34.3
23	7.49	35.5
24	8.47	35.0
25	7.94	35.5
26	8.40	35.3
27	6.05	35.2

Table 4. Comparison of SAI and TL_{50} of eggs between the second and third (final) spawning period. The values of SAI were obtained in the same manner as described in Table 1. Note the decrease of SAI and TL_{50} of eggs spawned in the final period.

Spawner	Date	SAI	TL_{50}	Spawning period
A	8.23	8.45	34.4 °C	second period
A	9.16	4.98	31.7	final period
D	8.13	6.61	33.3	second period
D	9.19	4.89	30.8	final period

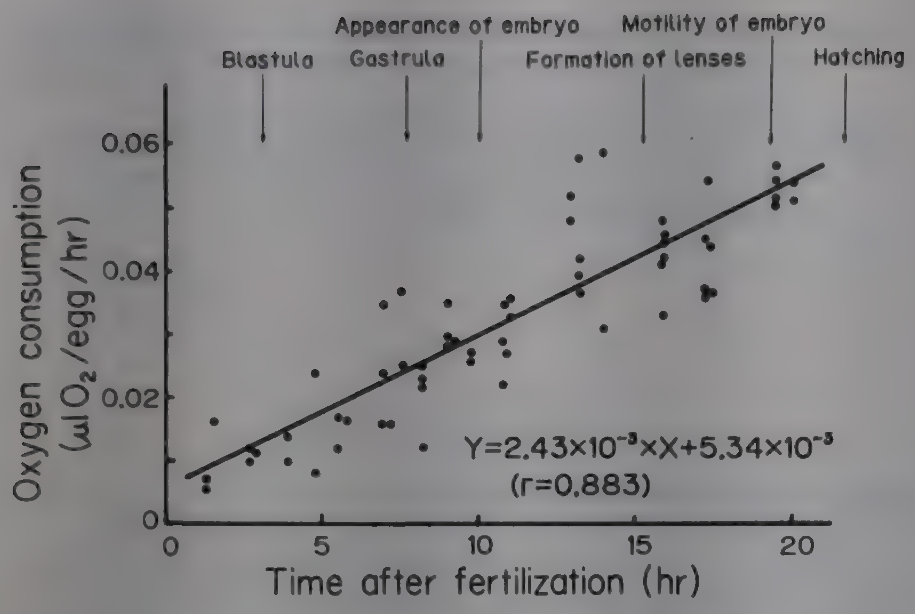

Fig. 1. Oxygen consumption rate of *Sillago japonica* eggs at 26°C.

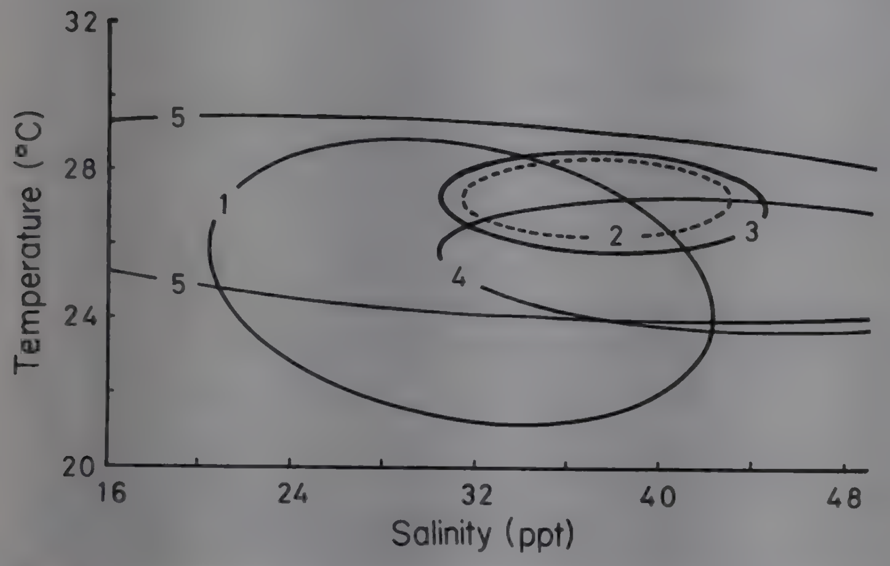

Fig. 2. Comparison of optimum temperature-salinity conditions for normal hatching in five different cases. Solid lines encircling the range show more than 90% normal hatching while the encircling dotted line shows 70%. Each number corresponds to that in Table 1.

Induced Spawning of *Clarias macrocephalus* (Gunther)

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SAIDIN, T. 1986. Induced spawning of *Clarias macrocephalus* (Gunther), p. 683-686. *In* J.L. Maclean, L.B. Dizon and L.V. Hosillos (eds.) The First Asian Fisheries Forum. Asian Fisheries Society, Manila, Philippines.

Abstract

Clarias macrocephalus does not spawn in captivity. Various hormones were used on mature female broodstock to determine the optimum dosage for natural oviposition or spawning. Four different agents were assayed at varying dose levels. Homoplastic pituitary extract dosages of 1.5 and 2 units (No. units = wt. donor fish/wt. recipient fish) were effective to induce natural oviposition. Heteroplastic pituitary extract from *C. batrachus* showed a similar pattern. *Pangasius sutchi* pituitary extract injection was effective at 4 and 6 units, with 4 units being more significant. Human Chorionic Gonadotrophin was effective at 300-400 IU/100 g body weight of female. The analog of leutinizing-releasing hormone (LRH-A) was successful at 1µg, 2µgand 3µg per 100 g body weight of female, with levels of 2 µg100 g body weight the most effective dosage.

Introduction

Clarias macrocephalus, locally known as Keli Bunga or Keli Mamah, flourishes well in inundated paddy fields during rainy seasons. The breeding season coincides with the rainy season. In captivity, this catfish exhibits refractive behavior. Maturity is attained after about 10-12 months of culture period. Induced spawning of *C. macrocephalus* using pituitary extract has been reported in Thailand (Tongsanga et al. 1963; Sidthimunka et al. 1968) and in the Philippines (Carreon et al. 1973).

Since 1981, the Freshwater Fisheries Research Institute, Batu Berendam, Melaka, has undertaken research in the induced spawning of *C. macrocephalus*. In 1982, this Institute succeeded in spawning *C. macrocephalus* using homoplastic and heteroplastic pituitary extract. In 1983, the Institute successfully used Human Chorionic Gonadotrophin (HCG). The most recent achievement was successful use of LRH-A (an analog of Luteinizing Releasing Hormone donated by the Bureau of Fisheries, Taiwan) in induced spawning of *C. macrocephalus*.

Artificial oviposition by stripping has resulted in quite low hatching rates 10-45%, while natural oviposition produces about 75% hatching rate. This is in agreement with observations made by Carreon (1976) where natural oviposition produced between 21 and 81% with average hatching rate of 58%. Artificial stripping has an added disadvantage, in that the male has to be sacrificed for the extraction of the milt. Therefore, the use of natural oviposition was attempted in this study.

Materials and Methods

Broodstock were conditioned separately for at least two days to allow for emptying of stomach content. The following day, they were selected and weighed. Only females with loose extruding eggs when slight pressure was applied on the belly, were selected. Fourteen females were sampled to determine the gonadosomotic index (GSI) and absolute fecundity. Absolute fecundity is the total number of eggs available in the gonad. It is determined by counting the number of eggs per gram weight of gonad and multiplying this number by the total weight of the gonad. Relationships between body weight, gonad weight, GSI and mean absolute fecundity were compared using correlation matrix.

Tanks and *hapas* (net enclosures) for breeding were prepared. Substrates such as *Eichhornia* plants were placed in all the tanks and *hapas*. PVC pipes of 12-cm diameter were also provided as a hiding place.

Homoplastic and heteroplastic pituitary glands and other hormones were obtained prior to the spawning activity. All injections were done intramuscularly. For homoplastic pituitary extracts, pituitary glands of matured *C. macrocephalus* were collected, and if not used directly, preserved in acetone with two changes and stored in a refrigerator at temperatures of 0-2°C. Three dose levels were used: 1, 1.5 and 2 units, each with four replicates. Males were given 1 unit each. The number of units is defined as the ratio: weight of donor fish ÷ weight of recipient fish. Each gland was first homogenized manually before injection of the extract.

For heteroplastic pituitary extracts, pituitary glands of *C. batrachus* were collectd as above. Three dose levels were used: 1, 1.5 and 2 units each with four replicates. Males were given 1 dose each. Also, pituitary extracts of *Pangasius sutchi* were prepared. Three levels of dosage were used on the females: 2, 4 and 6, each with four replicates. Males were given 2 units each.

Purified Human Chorionic Gonadotrophin (HCG) was used. Six dose levels were tried on females: 150, 200, 250, 300, 350 and 400 IU/100 g, each with four replicates. Males were given 150 IU/100 g.

Four dose levels of Luteinizing-Releasing Hormone (LRH-A) were tried: 1, 2, 3 and 4μg/100 g, each with four replicates. Males were given 1μg/100 g of LRH-A.

The Chi-square test of significance was used to determine the optimum effective doses. The latent period or the time when oviposition occurred was recorded. The working fecundity, defined as the total number of eggs released during the process of artificial oviposition (stripping), was determined for some of the ovulated females. The percentage hatching was also determined for some of the ovulated females. Verification trials based on the effective dosages were conducted to prove their efficacy.

Results and Discussion

Results of the experiments are contained in Tables 1-5. The fecundity of matured *C. macrocephalus* females weighing 177.8-368 g is in the range of 18,650 to 34,640. Statistical analysis of the data showed that there was a positive correlation between all the parameters measured except for body weight and GSI, which showed a negative correlation. However, comparisons on the correlation matrix (Table 2) revealed that the relationship between gonad weight and mean absolute fecundity was highly significant ($P < 0.01$). Subsequently, the relationships between body weight-gonad weight and body weight-mean absolute fecundity were also significant ($P < 0.05$).

In the induced spawning experiment using various inductive agents, *C. macrocephalus* responded positively towards injections of homoplastic and heteroplastic pituitary extracts, as well as with gonadotrophin and an analog of luteinizing-releasing hormone. For both homoplastic and *C. batrachus* pituitary extracts, similar results were observed on the percent ovulated females and the range of percentage hatching (Table 3). Chi-square comparisons (Table 4) between the two levels of dosages for each hormone showed that the two levels were significantly different ($P < 0.05$). Hence, based on the percentage of ovulated females a dosage of 2 units was more effective than 1 unit, even though their percentage of hatching showed no significant difference. Trials conducted for verification produced consistent results (Table 5). Similar observation was also observed for injection using *P. sutchi* pituitary extract with 4 units as the most effective dosage.

The administration of HCG at various dose levels showed that three dose levels were found to be effective. They are 300 IU/100 g, 350 IU/100 g and 400 IU/100 g. Chi-square comparisons between the percentages of ovulated females for the three dosages showed that they were not significantly different (Table 4). Verification trials (Table 5) also showed that these three dosages were equally effective in inducing natural oviposition. However, for cost effectiveness, only 300 IU/100 g and 350 IU/100 g would be regarded as the optimum effective dosage.

Subsequent trials using LRH-A showed that dose levels of 1μg/100 g and 2μg/100 g were effective in terms of the percentage of ovulated females. However, chi-square comparisons on the percentages of ovulated females showed that they were highly significance different ($P < 0.01$). Hence 2μg/100 g could be regarded as the most effective dosage for LRH-A.

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Table 1. Mean fecundity and GSI of *C. macrocephalus*.

Wt. of female (g)	Wt. of gonad (g)	GSI	Range of absolute fecundity n = 4
292.0	51.20	17.53	33,500 – 35,780
236.6	35.80	15.13	22,127 – 24,927
228.9	28.05	12.25	17,100 – 20,300
337.0	33.10	9.82	24,428 – 27,548
358.0	40.8	11.40	26,830 – 30,290
368.0	51.5	14.00	32,269 – 36,389
248.8	35.6	14.31	22,887 – 26,537
223.8	32.6	14.52	18,759 – 21,455
227.8	26.9	11.81	18,669 – 22,193
177.8	25.4	14.28	17,516 – 19,784
203.3	25.6	12.59	17,756 – 20,250
197.3	32.7	16.57	20,387 – 23,893
271.3	36.9	13.60	21,992 – 25,742
217.8	36.3	16.67	23,563 – 26,871

Table 2. The relationships between body weight, gonad weight, GSI and mean fecundity using correlation matrices.

Parameters	Gonad weight	GSI	Mean fecundity
Body weight	0.71^{x}	-0.36^{ns}	0.79^{x}
Gonad weight	–	0.37^{ns}	0.97^{xx}
GSI	–	–	0.24^{ns}

x – significant, $P < 0.05$
xx – highly significant, $P < 0.01$
ns – not significant

Table 3. Success rate of induced spawning of *C. macrocephalus* using various inductive agents.

Type of hormone	Dose Male	 Female	No. ovulated (%)	Estimation of the range of percentage hatching	Mean working fecundity
1. Homoplastic pituitary extract from *C. macrocephalus*		1.0 unit	nil	–	
	1 unit	1.5 units	50	45-70	9,081
		2.0 units	100	50-75	13,776
2. Heteroplastic pituitary extract					
a. *C. batrachus*		1.0 unit	nil	–	
	1 unit	1.5 units	50	40-75	14,285
		2.0 units	100	50-75	13,400
b. *P. sutchi*		2.0 units	nil	–	
	1 unit	4 units	100	40-70	14,380
		6 units	50	40-60	8,970
3. HCG		150 IU/100 g	nil	–	
		200 IU/100 g	nil	–	
	150 IU/ 100 g	250 IU/100 g	nil	–	
		300 IU/100 g	75	60-70	14,760
		350 IU/100 g	100	60-70	14,920
		400 IU/100 g	100	50-70	15,662
4. LRH-A		1 µg/100 g	50	30-50	10,172
	1 µg/ 100 g	2 µg/100 g	80	40-60	14,766
		3 µg/100 g	25	20-50	3,450

Table 4. Chi-square comparisons on the percentages of ovulated females, and percentages of hatching.

Sequences	Percentage of ovulated females	Percentage of hatching
1. Homoplastic (1.5 units) (2.0 units)	16.67^{x}	7.50^{ns}
2. Heteroplastic a. *C. batrachus* (1.5 units) (2.0 units)	16.67^{x}	5.93^{ns}
b. *P. sutchi* (4 units) (6 units)	16.67^{x}	22.73^{xx}
3. HCG (300 IU/100 g) (350 IU/100 g) (400 IU/100 g)	4.54^{ns}	17.80^{x}
4. LRH-A (1 µg/100 g) (2 µg/100 g) (3 µg/100 g)	50.00^{xx}	5.83^{ns}

x — significant at $P < 0.05$
xx — highly significant at $P < 0.01$
ns — not significant

Table 5. Verification trials on the success rate of induced spawning using the effective dosages.

Types of hormones	Effective dosage Male	Female	No. of females	Percent ovulated	Estimation of range of percentage hatching
1. Homoplastic pituitary extract of *C. macrocephalus*	1 unit	1 unit	8	40	40-60
		2 units	8	75	50-70
2. Heteroplastic pituitary extract a. *C. batrachus*	1 unit	1 unit	8	37.5	40-60
		2 units	8	75	50-75
b. *P. sutchi*	2 units	4 units	24	83	40-70
		6 units	8	62.5	30-50
3. HCG	150 IU/ 100 g	300 IU/100 g	40	87.5	60-80
		350 IU/100 g	20	80	60-80
		400 IU/100 g	8	87.5	50-70
4. LRH-A	1 µg/ 100 g	1 µg/100 g	8	50	50-70
		2 µg/100 g	8	87.5	50-70

The latent period between injection and natural oviposition was between 16 and 18 hours at water temperature of 26.5 to 27°C.

Induced Spawning of *Pangasius sutchi* (Fowler) Using an Analog of Luteinizing Releasing Hormone and Homoplastic Pituitary Extract

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Abstract

Pangasius sutchi, a riverine catfish, did not respond to spawning induction using single injection of an analog of luteinizing releasing hormone (LRH-A). However, when injected with two sequences of injections at varying dose levels, 33% ovulated with a stimulatory dosage of 20μg and resolving dose of 30μg LRH-A/kg. Dosages lower and higher produced negative results. Trials using LRH-A in combination with homoplastic pituitary extract (HPE) showed promising results. A stimulatory dose of 1.0 unit of HPE plus 10μg LRH-A/kg followed by a resolving injection of 1.5-2.0 units of HPE plus 20-30μg LRH-A/kg produced 79-85% ovulation. (No. HPE units = wt. donor fish/wt. recipient fish).

Introduction

Sexually mature *P. sutchi*, a riverine catfish, can be induced to spawn using homoplastic and heteroplastic gonadotropin hormones, either singly or in combination (Thalathiah et al. 1983).

In an effort to furnish information on the response of different types of hormones on *P. sutchi*, an analog of luteinizing hormone-releasing hormone (LH-RH) was chosen. LRH-A is a synthetic nanopeptide, and an analog of LH-RH obtained from Taiwan. It has proven to be highly effective in the induction of spawning in cyprinids, including *Hypophthalmichthys molitrix*, *Aristichthys nobilis*, *Ctenopharyngodon idella* and *Mylopharyngodon piceus* (Anon. 1977a, 1977b). The minimum effective dosage of LH-RH is 1μg/kg with a spawning success of 86.3% in grass carp. Its ability to induce successful spawning in several species has already been reported in goldfish (Lam et al. 1975, 1976); Japanese medaka *Oryzias latipes* (Chan 1977); ayu *Plecoglossus altivelis* (Hirose and Ishida 1974); and plaice and goby (Aida et al. 1978).

There is no published report on the success rate of induced spawning of *P. sutchi*. In this study an attempt was made to evaluate the spawning success of *P. sutchi* using LRH-A analog, either singly or in combination with homoplastic pituitary extract.

Materials and Methods

Mature gravid males and females were selected from broodstock ponds by netting during the breeding season, mid-April to mid-June, and September and December-January (Thalathiah et al. 1983). The breeders were taken to the hatchery in fiberglass tanks, weighed and tagged. Ova were cannulated and brought to the laboratory for determination of maturity stage by clearing in 30% alcohol, 50%, 70%, 90% and absolute alcohol for five minutes each, followed by xylene. Ova with average diameters of 1 mm and of uniform size with distinct nuclei were taken as the tertiary stage and were used for induced spawning.

Preliminary trials were conducted with a single injection of LRH-A. The dose levels were 10, 20, 30 and 40μg/kg, all with replicates. The fish did not respond.

Subsequent trials were conducted by administering two sequences of LRH-A at a time interval of 8 hours. The dose level for resolving injection was much higher than the dose level for stimulatory injection. These levels (see Table 1) were selected based on the earlier experiments using single injection.

A third trial was conducted using LRH-A in combination with homoplastic pituitary extract (HPE) collected prior to spawning activity. This was based on the experience that *P. sutchi* females respond positively to induction using a combination of HPE and human chorionic gonadotropin (Thalathiah et al. 1983). All injections were administered intramuscularly.

Doses of HPE were given as "units" where the number of units was the ratio: wt. donor fish/wt. recipient fish.

The administration of hormones was divided into two sequences of injections, stimulatory and resolving, as shown in Table 2. Artificial oviposition or stripping of eggs was done about 10-12 hours after the resolving injection. The dry method of fertilization was adopted. Eggs were incubated in hatching funnels with running water. Hatchlings were collected in trays and later transferred to a hatchery for rearing.

Results and Discussion

Results of the experiments are given in Tables 1 and 2. In the trial using two injections of LRH-A, only the dosage of 20µg/kg plus 30µg/kg resulted in ovulated females (Table 1). Examination of the gonads showed that at dosages of 10µg plus 50µg/kg per kg and 20µg plus 40 µg/kg, ova were regressed and hydrated. This could be due to the high dosage of LRH-A administered which was equivalent to 60µg/kg.

The treatment of LRH-A in combination with homoplastic pituitary extract also resulted in spawning. The first dosage (1.0 units of HPE plus 10µg LRH-A/kg and 1.5 units of HPE plus 30µg LRH-A/kg) produced 67-100% ovulation (Table 2). The second dosage (1.0 units of HPE plus 10µg LRH-A/kg and 2.0 units of HPE plus 20µg LRH-A/kg) produced between 33%-100% ovulation. The t-test showed that the two treatments were not significantly different. The effectiveness of the combined LRH-A and HPE was much higher than the combined HCG and HPE in terms of working fecundity.

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Table 1. Induced spawning of *P. sutchi* with two sequences of injections of LRH-A at various dose levels.

Stimulatory (µg/kg)	Resolving (µg/kg)	No. of replicates	No. spawning	Percentage hatching
10	30	3	0	
10	40	3	0	
10	50	3	0	
20	30	3	1	60
20	40	3	0	

Table 2. The success rate of induced spawning of *P. sutchi* using LRH-A in combination with HPE.

Stimulatory: 1.0 D* HPE + 10 µg LRH-A/kg Resolving : 1.5 D HPE + 30 µg LRH-A/kg		Stimulatory 1.0 D HPE + 10 µg LRH-A/kg Resolving 2.0 D HPE + 20 µg LRH-A/kg	
No. of replicates	No. spawning	No. of replicates	No. spawning
3	2	3	3
4	4	4	3
4	4	3	1
4	3	3	2
3	3	4	3
4	3	4	4
Mean	86.17	Mean	79.17

*D signifies Dose = Weight of donor ÷ weight of recipient.

Spawning occurred 10-12 hr after the resolving injection at a water temperature of 27°C. Hatching occurred 18-22 hr after fertilization at water temperatures of 26.5-27°C.

Spawning Ecology and Behavior of the Mahseer *Tor putitora* (Hamilton) in the Himalayan Waters of Nepal

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Abstract

The breeding ecology and behavior of mahseer *(Tor putitora)* of the Himalayan waters was investigated in Tadi River, a feeder stream of the Trisuli River, Nepal. The spawning grounds of the mahseer in the river are 2 to 2.5 m deep with sandy bottom, pebbles and aquatic weeds and with temperature and dissolved oxygen higher than in the other parts of the river. The spawning season extends from June to September. Intermittent breedings and the size of the fish at maturity were recorded. Some physicochemical parameters influencing breeding are discussed.

Introduction

Many investigators have studied the breeding ecology of food fishes of game value while a few have carried out intensive studies on mahseers. Beavan (1877), Hora (1939, 1940), Khan (1939), Kulkarni (1971), Jhingran and Sehgal (1977). Pathani (1977) and Thomas (1983) made some observations on breeding. This study deals with spawning ecology, behavior, spawning duration and spawning ground, maturity size and ova diameter during the breeding season of mahseers in the running waters of Nepal.

Materials and Methods

Live specimens of adult mahseer *(Tor putitora)* (Fig. 1) were collected by trammel nets and gill nets. A survey of the spawning ground was carried out with long-handled lift nets and gill nets. The color and features of fertilized eggs in natural spawning dens were identified by regular observations. Dissections of various fish were done to determine maturity stages. Physicochemical parameters such as water quality, dissolved oxygen and pH were determined by using standard ecological methods (Table 1).

The period of spawning as inferred by the presence of mature eggs and sperm in the body cavity was confirmed by finding spawn, fry and fingerlings in the river. The ova, attached to stones in the breeding place, were collected and counted. The breeding ground of the mahseer was determined by the presence of eggs and fry and of broodfish engaged in sex play. The temperature, gradient, current and dissolved oxygen in the breeding ground were recorded. The samples were collected both from snowfed and rainfed streams.

Results

Spawning grounds. The Tadi River spawning area is situated about 70 km northeast of Kathmandu at an altitude of 500 m. The depth is 1-15 m with the deepest at 30 m. The shallow water river bank varies from 2 to 5.5 m in depth, and it is here where the mahseers spawn. The width of the river is 75-140 m.

Tor putitora spawn from June to September in the snowfed Tadi stream and its rainfed tributaries. In these months water velocity, temperature, pH and dissolved oxygen are favorable for breeding (Table 1). In the spawning period the river becomes turbid due to the melting of snow at the mountain peaks. Mahseers move from the deeper waters of the lower regions of the Trisuli River and reach the upland spawning ground of Tadi River to breed (Fig. 2). The upriver movement usually begins in July, when water started to become turbid and the river was flooded by the influx of the rain. The mahseer breeding pair select shallow semi-stagnant water along the river bank interspersed with gravel and stones. The eggs attach to stones in batches. Ideal spawning grounds were located mostly in the Tadi River near Gadhkar-Chokedovan and at the confluence of the Tadi and Trisuli Rivers at Deughat. Spawning grounds were also located in Kharae Khola, a feeder stream of Tadi River. Characteristics of spawning sites were observed throughout the study (Table 2).

Experimental gill netting within 2.5-5.5 m depth was carried out at night during the breeding season. Some aquatic weeds of the region collected were *Ceratophyllum, Myriophyllum, Hydrilla, Vallisneria, Polygonum* and *Potamogeton*. Mahseer eggs and larvae were also collected from 2-3.5 m depth by a long-handled dip net or "Ghorleng" and their presence indicated a spawning site. However, breeding grounds extended up to 5.5 m depth in certain places. Eggs were sometimes collected attached to *Eloda* leaves, but most of the eggs and larvae of *T. putitora* were collected free along with sand and gravel at the bottom. From the same area broodfishes were caught by large trammel nets at night and were actually seen to spawn at midnight.

Spawning behavior. Courtship in mahseer is a long process starting when several, usually seven, males chase the females. When the female finds a suitable site, the eggs are laid and at the same time the males swim twisting around the female, touching it by making brisk movements of the caudal region, after which they fertilize the spawned eggs with their milt. After a short pause, both males and females return to the deeper area of the pool, neither of them exhibiting parental care. The male and female mahseers could easily be identified in shallow water regions by their sexual characteristics and size, the males always smaller than the females.

Spawning cycle. Dissection of male and female *T. putitora* revealed that the spawning period is June-September. At spawning the female exudes eggs and the male oozes milt when pressure is applied on the abdomen. After the second week of September the gonads were spent. These observations were also confirmed by the developing eggs and larvae collected during the first week of September. The ova diameter at this period ranged from 1.5 to 2.85 mm (Table 4). Ripe ovaries of *T. putitora* showed three groups of eggs: small, medium and large (Table 4). The smallest size group of eggs were very few in August and September as they had developed further, indicating three acts of spawning. Therefore, mahseers are intermittent breeders capable of multiple spawning during breeding periods.

Characteristics of eggs and fry. The spawn or fertilized eggs collected from spawning sites were yellow and slightly orange. The fertilized egg measured 2.18-2.87 mm (Table 3) and hatched after 49 hours into yolksac fry which persisted for about 160 hours, when full fledged larvae were formed. The reared fry, 12 days after hatching in the Gadkhar farm, acquired adult characters. The yolk had completely disappeared after 168 hours. The snout and head chromatophores also disappeared in 192 hours but many chromatophores were still scattered on the back of the larvae. After 10 days fins were fully formed, the pectoral fins with 9-10 rays and the anal fin with more rays. A spot on the caudal peduncle was very prominent.

Maturity size and age of *Tor putitora* were deduced. Males matured ahead of females at 200 mm total length at two years of age; females matured at 300 mm total length within or after three years.

Discussion

The first authentic account on spawning habits of mahseers was recorded by Thomas (1983) who observed that eggs were laid in batches and that the pelvic and anal fins were used during the spawning act by the male fish to mark out a redd in gravelly ground. In Nepal, *T. putitora* apparently laid out three batches of eggs in one breeding season on the gravelly or sandy spawning sites with considerable hollowing or constructing of the redd.

Beavan (1877) observed small batches of eggs laid for several months (May-August) by mahseer, while Hora (1940) recorded that the breeding season of *T. tor* was from August to September. Cordington (1946) reported variable breeding seasons of mahseer in different natural waters. Khan (1939) recorded that the mahseers of Punjab waters spawn thrice a year in the spawning grounds with small stones and pebbles in shallow regions with sand. Smith (1947) reported that mahseers breed throughout the year. But in this study, mahseers in Nepal were found to breed from June to September only.

Kulkarni (1971) recorded that the breeding season of *T. khudree* was July-August when high floods and low temperature prevailed. As with *T. khudree* the impact of floods was seen in the spawning of Nepalese mahseers but higher water surface temperatures prevailed in the spawning areas. Desai (1973) observed that the breeding season of *T. tor* (Narvada River) was July-March and that fish attained first maturity after 360 mm total length with four batches of eggs in the mature ovary.

Chaturvedi (1976) reported that the smallest mature male of *T. tor* from Udaipur lake was 254 mm and all males matured after 310 mm length, while the smallest mature female was 322 mm and all females matured after 390 mm length within one year. Qasim and Qayyum (1961) observed that *T. putitora* from Aligarh waters spawned several times over the greater part of the year having batches of eggs at all stages of maturity in the ripe ovary. The sizes of ripe eggs in mahseer were variable which may be due to the condition of the fish in the river, as stated by Nikolsky (1963) and Pathani (1982). In Nepalese masheers all immature eggs were light orange yellow and not transparent in contrast to varied colored eggs in the ripe ovary of *T. khudree* as reported by Kulkarni (1971).

The spawning behavior of Himalayan fishes is not clearly known (Shreshtha 1979). The spawning of snow trout *(Schitzothorax plagiostomus)* in the running waters of Nepal depends upon suitable environmental factors, such as rainfall, flood, pH and temperature (Shreshtha 1979). The effects of these factors on mahseers of Nepal are not yet understood.

It appears that the flooded streams are richly charged with mineral ions and large amounts of dissolved oxygen, and have relatively warm surface waters (at the confluence of the stream and river), clear water, teeming abundance of insect food and absence of harmful predators. These may be some of the factors responsible for the upriver spawning migration of mahseer which takes place during high flood at midnight and always coincides with the full moon. Perhaps moonlight penetration of the stream floor helps in the recognition of the sexes and provides excitement for sex play and courtship acts.

The function of flood in the reproductive activities of fishes is not clearly known (Shrestha 1981). The turbid waters of flooded streams are not apparently a deterrent to spawning although great turbulence due to rapid flooding as in July and August is unfavorable and mahseer breed with decreasing intensity at high flood periods. It appears that well-oxygenated, gently advancing flood water over the gravel beds provides the necessary rheotactic stimulus, evoking sexual play in *T. putitora*. A newly inundated stream bed with flood is less likely inhabited by enemies and predators like fry-eating insects and amphibians and there is less chance of fungal and bacterial infection of the developing egg and fry. Therefore, mahseers leave their parent river for spawning grounds of feeder streams to breed. After the onset of the flood is over, they move downriver to rejoin the main river channel.

The rain water and snow melt water appear to play a vital role in the regulation of the reproductive activity of the Himalayan fishes. Both rainfed and snowfed flood waters are always charged with inorganic ions and have high specific conductance. Such waters may provide a stimulus for the gonadal maturation and spawning. In this study spawning in the mahseer was found to be correlated with sustained rainfall and melting snow.

Everywhere in Nepal the mahseer fishery is poor. Fishermen throw dynamite into the water and strike rocks with hammers to stun fingerlings of mahseer sheltering underneath the rock crevices. Migratory brood populations must be conserved and sufficient numbers of adult spawners be allowed to escape the fishery (trammel nets) and spawn in a clean and undamaged environment. If such opportunities are provided for the wild migratory population, surges of mahseer will reappear in the large rivers and hill streams.

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Table 1. Water quality of Mahseer spawning ground, Tadi River.

Temperature	30° c.
Color	Brown
Visibility (m)	0.38
Compensation depth	0.85
pH	7.8
Oxygen ppm	19.5
Dissolved solids (mg/l)	160.5
Hardness	48.0
Specific conductance	584.0 mohs
Chloride mg/l	15.5
Sulphate mg/l	12.5
Calcium	28.0
Magnesium	3.0
Iron	0.18

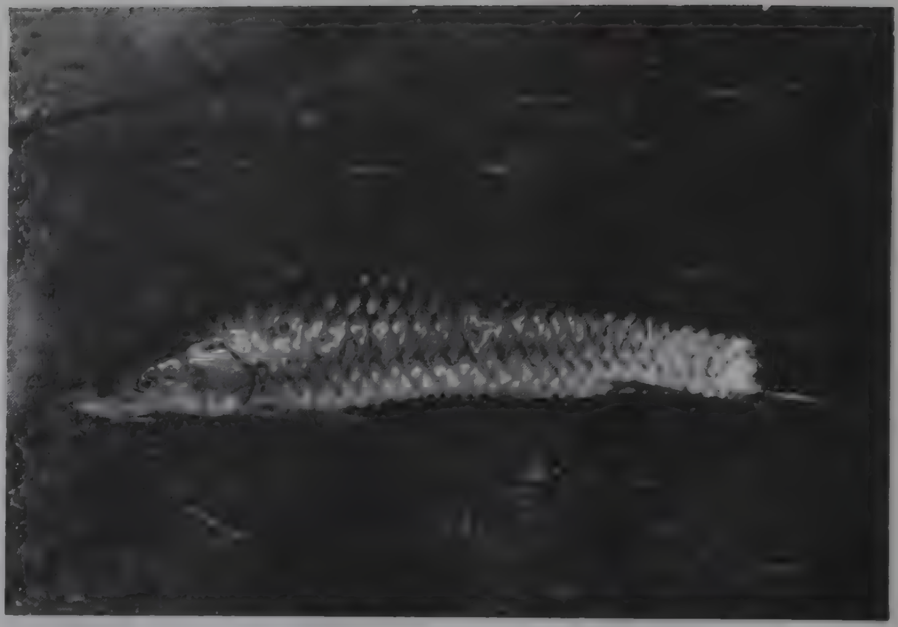

Fig. 1. Golden mahseer *(Tor putitora)*.

Table 2. Distribution of mahseer eggs in spawning ground, Tadi River.

Spot no.	Nature of substratum	Number of attached eggs
1	Rocks and stones	118
2	Rubble	145
3	Gravel	275
4	Fine sand and silt	100
5	Logs and debris	55

Fig. 2. Spawning beds of mahseer, in the Trisuli River. Mahseer ascends from large rivers to small creeks to spawn. They usually select spawning grounds near river confluenes.

Table 3. Fertilized eggs of *Tor putitora* from Trisuli River.

Months	Number of egg trail (average)	Fertilized egg dia (mm)
June	20	2.18
July	25	2.80
August	50	2.85
September	160	2.87
October	15	2.60

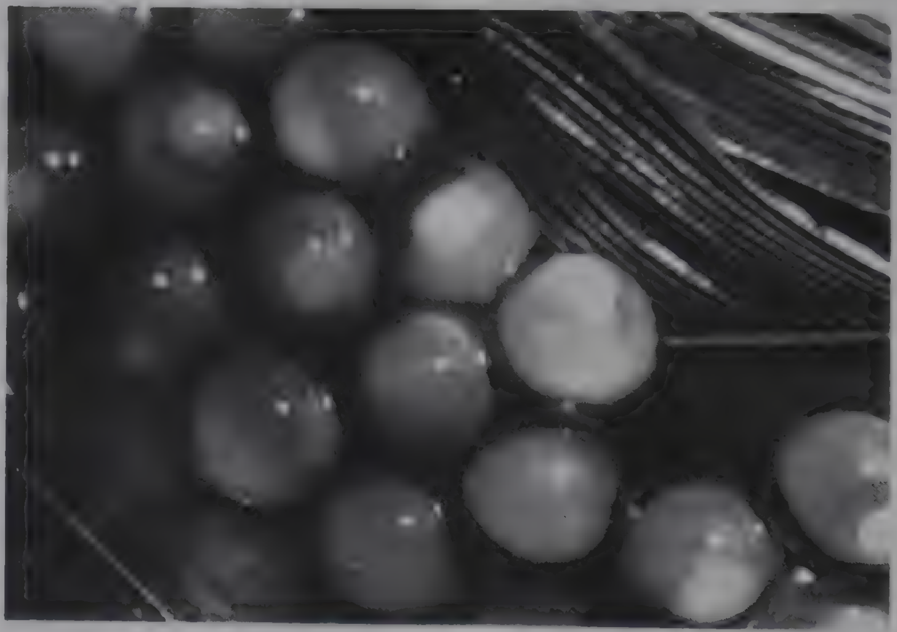

Fig. 3. Fertilized eggs of mahseer (about 30 hr) collected from spawning beds.

Table 4. Sizes of ova in *Tor putitora*.

Months	Reserve stock of immature eggs (mm)	Opaque mature eggs (mm)	Transparent fully-mature eggs (mm)
June	0.500	0.900	1.950
July	0.510	0.950	1.990
August	0.700	0.990	1.800
September	–	1.850	2.800
October	–	1.500	2.850

Reproductive Strategy of *Labeo dussemerii* and Implications of Hydroelectric and Irrigation Projects on the Mahaweli Ganga, Sri Lanka

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Abstract

The Hirikani, *Labeo dussemerii*, forms the major part of the catch of the lowland section of the Mahaweli Ganga, the largest river in Sri Lanka.

The flow pattern of the Mahaweli is typically bimodal with peak discharge and flooding during the northeast monsoon (November-January) and a lesser discharge peak during the southwest monsoon (May-June). The majority of fish spawn at the time of increased discharge following the August/September dry season. Gonadal development takes place in June and July followed by a "quiescent phase" during the August-September dry season. The spawning season is limited to October and November prior to the major floods in December. Approximately 10% of the population spawned in June during the minor discharge peak. The smaller percentage that spawned during the minor discharge peak probably reflects a lower production potential of the river at this time compared to the time of major floods. The completion of three major dams in the upper catchment of the Mahaweli will radically alter the downstream areas. It is predicted that this will lead to a decrease in the northeast monsoon spawners, thereby decreasing the overall population and production of this species.

Introduction

Changes in temperature and daylength have been shown to trigger reproductive behavior in physiologically-primed fish in temperate regions and appear to be synchronizers of gonadal development and reproductive behavior in the higher latitudes of the tropics (Scott 1979). However, other environmental and biotic factors may determine the reproductive behavior in equatorial fish, as the seasonal differences in daylength and temperature diminish with decrease in latitude. Annual fluctuation in rainfall appears to be the dominant environmental influence on the reproductive cycle of tropical and equatorial freshwater fish. Rainfall in equatorial regions, although less seasonal than in the tropics, is still characterized by equinoctial peaks, which generate the typical bimodal flow pattern of equatorial rivers. Many riverine species spawn during times of peak discharge (Lowe-McConnell 1979; Welcomme 1979).

This study investigates the environmental synchronization of the reproductive cycle of an equatorial cyprinid, *Labeo dussemerii* (Valenciennes), that inhabits the Mahaweli Ganga of Sri Lanka, where three major dams have recently been completed in the upland catchment areas as part of the Accelerated Mahaweli Ganga Hydroelectric Irrigation Project (Aker 1985). These investigations, started in 1981, were completed by April 1983 before the above constructions had any major effect on the seasonal discharge pattern of the lowland section of the river. In common with other *Labeo* species throughout Africa and Asia, *L. dussemerii* is the dominant catch of the lowland riverine fisheries in Sri Lanka.

The Mahaweli originates in the central highlands and runs 335 km through the lowlands of the dry zone (Arumugam 1969). Rains in the upper catchment are equally heavy during both monsoons, whereas the downstream section receives most of its rain during the northeast monsoon (Figs. 1 and 2). The main site was a section of an anabranch of the Mahaweli named the Periya Aru (Fig. 1). This downstream lowland section has the characteristic bimodal flow pattern of an equatorial river but is atypical in its extreme fluctuations (Fig. 2). Simultaneous high rainfall in upland and lowland catchments in November-January causes complete inundation of the lowland. The increased discharge from the upland rains of the southwest monsoon in May-June is sufficient to flood the downstream marshlands adjacent to the river but not the slightly more elevated forest and farmland of the lowland.

Materials and Methods

Visits to the site were made almost monthly in 1981-1983. The movement of fish was studied by setting 5-cm and 10-cm mesh gill nets at selected points (Fig. 1) and by casting nets all over the area. Fork length and body and gonad weight were determined. Frequency distribution of oocytes by size (diameter) and fecundity, defined as the number of eggs produced by the mature female just prior to spawning, were measured by wet sub-sampling of eggs preserved in Gilson's fluid (Bagenal 1968). Length-frequency analysis (Cassie 1954) was carried out on a large number of fish caught in April 1982. The diurnal range of water temperature was measured with maximum/minimum thermometers and light intensity in the water column with a Griffen Environmental Comparometer.

Results

Light penetration and water temperature showed well-defined minima during high discharge (Fig. 3a). The greatest diurnal changes in temperature occurred at the height of the dry season (with maxima of 35.5ºC and minima of 29ºC in exposed areas and with maxima of 33.5ºC and minima of 29ºC in shaded areas). As discharge increased, the mean temperature and the diurnal range in temperature decreased to a maximum of 27ºC and a minimum of 25ºC at the higher discharge rates. The reduction of light penetration with increased discharge was such that during floods less than 5% of the residual light could be detected at 0.3 m. During the dry season up to 70% of the residual light could still be detected at 0.3 m except in some exposed areas where phytoplankton bloom reduced it to 40%.

At the end of the dry season *L. dussemerii* were confined to the shaded deep pools of the Periya Aru. As soon as discharge increased, fish were found swimming upstream towards the Mahaweli. During the flood *L. dussemerii* dispersed laterally and were caught in the flooded forest areas between the Periya Aru and the main Mahaweli channel. As the flood waters receded *L. dussemerii* were found throughout the Periya Aru and Mahaweli waters. However, they were found in the floodplain marshland only when it was inundated with river water and its limnological characteristics were similar to those of the river water. Size-frequency analysis of fish caught in early April 1982 identified three length classes of 110 ± 10 mm, 227 ± 12 mm and 288 ± 16 mm.

The relationship between fork length, gonad weight, somatic weight and fecundity in *L. dussemerii* (Table 1) indicates that gonadosomatic index (GSI = gonad weight/somatic weight) increased with weight. In the majority, the most rapid gain in GSI occurred in July-August before the onset of the severest part of the dry season (Fig. 3b). GSI remained stable throughout the September dry season until spawning. Spent fish were caught three days after the initial increase in river flow in late September but mature fish were still found up to early November. All fish were spent when the forest areas were flooded in December. The distinct modes in the GSI of the July female fish (Fig. 3b) imply that fish spawning in late November mature later than those spawning during the initial floods in September. A small number of fish (< 10%) caught in August were immature and a similar proportion of the catch in April had maturing gonads. The length of the maturing fish in April (256 ± 5, 292 and 325 mm) did not conform to the length classification of the whole population. This discrepancy suggests the existence of a subpopulation that spawned during the minor floods of May-June. Approximately 90% of the wet weight of the ovary and 70% of the testis were released on spawning.

The distribution of oocytes of varying diameters over a range of ovary sizes at different times of the year (Fig.3c) showed a quiescent ovary in March with no oocytes over 0.9 mm. Oocytes began to enlarge in June and appeared as a well-established mode of 1.0-1.3 mm diameter at the peak GSI in September. In October spent fish showed no oocytes above 0.9 mm, indicating that *L. dussemerii* is a total spawner. The relationships between various parameters and fecundity (defined as the number of oocytes above 0.9 mm) are given in Table 1.

Discussion

Spawning in the majority of *L. dussemerii* in the floodplain of the Mahaweli Ganga coincided with the increased discharge during the northeast monsoon, although a possible subpopulation appeared to have spawned during the minor discharge peak associated with the southwest monsoon. Biannual breeding has previously been reported among equatorial species in the Amazon and Zaire rivers which also exhibit bimodal floods (Roberts 1973). The October-November spawning took place in the river channel prior to the total inundation of the floodplain in December. The fry are therefore ensured of a food supply during their initial stages which increases as they grow. The small proportion that spawned during the May-June increase in discharge probably resulted from the less adequate food supply for the fry at that time compared to October-December when fish have temporary access to the forest floodplain. Increased river discharge was associated with a rapid fall in temperature and light intensity. As with temperate fish, light and temperature could be important in the spawning behavior of *L. dussemerii*.

The environmental factors that initiate gonad recrudescence in *Labeo* are uncertain. Recrudescence occurred during varying but low discharge with overall increase in temperature and light penetration. Gonad development takes place prior to the September dry season in October-November spawners. In contrast, it appears that in June spawners' gonads develop after the more extensive February-March dry season. As in Indian carps

(Parameswaren et al. 1970), gonad recrudescence in *L. dussemerii* appears controlled by an endogenous physiological rhythm which may be synchronized with environmental changes. *Labeo* caught in the Mahaweli in January 1981 and kept in outdoor flow-through tanks at Colombo University until August had the same gonadosomatic indices exhibited by fish caught in the Mahaweli in August. However, the captured fish did not spawn although kept until January 1982.

The three major dams in the highland catchment of the Mahaweli will certainly regulate the discharge rate in the lowland. The rate of release during the drier months will depend on hydroelectric-generating demand. The delay and attenuation of the discharge peaks in the rainy season will depend on the storage capacity of the dams at that time which in turn will be determined by the drawdown of the reservoirs in the previous dry season. The May-June discharge peak may disappear completely as its sources are mainly the upland rains and the three dams which impound much of the runoff after the extensive February-April dry season. The major October-December downstream discharge peak will probably be less affected as it follows a less extensive dry season and results from both upland and lowland rains. Discharge will be delayed and attenuated by the retention of upland runoff until the dams have filled to capacity.

The alteration of the natural flood cycle can affect the reproductive cycle of *L. dussemerii* in the Mahaweli in several ways. Initially, gonad development would continue as it does not appear to be dependent on environmental cues. However, the lack of food-induced environmental triggers in June will prevent spawning and therefore lead to the extinction of the minor subpopulation of June spawners. Spawning during the northeast monsoon may not necessarily be delayed by upstream impoundment if the local downstream precipitation and the increased discharge provide sufficient stimulation for spawning. However, if the discharge is decreased so as to prevent the flooding of the forested areas of the lowlands, the fry spawned during the northeast monsoon could have a more restricted and less productive environment and insufficient food for their first few months of life. This can result in higher mortality and eventually decrease the population and production of *L. dussemerii*.

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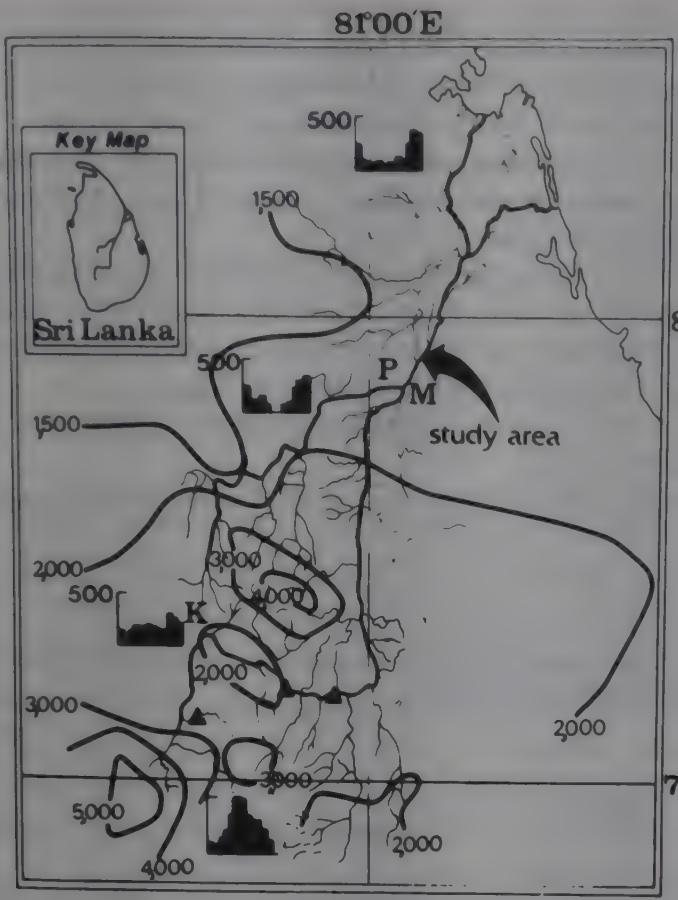
Table . The relationships between fork length (mm), somatic weight (g), gonad weight (g) and fecundity in *L. dussemerii* at peak gonosomatic index.

			r^2	P
Fork length (Fl) to somatic weight (sw)		: logSw=2.8logFl−4.3	0.94	0.001
Somatic weight to gonad weight (Gw)	♀	: Gw=0.29Sw−17.9	0.83	0.001
	♂	: Gw=0.065Sw−1.6	0.90	0.001
Fork length to fecundity (F)		: logF=3.8logFl−4.1	0.69	0.001
Somatic weight to fecundity		: F=602Sw−42980	0.76	0.001
Gonad weight to fecundity		: F=1845Gw+46	0.83	0.001


81°00'E
Key Map
Sri Lanka
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2000
P
M
K
study area

Fig. 1a. The Mahaweli Ganga Basin. Mean annual rainfall and seasonal distribution, mm. K and P denote the position of the highland (Katugastota) and lowland (Polonaruwa) rain gauges, respectively, and M, the river discharge gauging station at Mannampitiya bridge. ▲ Dam sites.

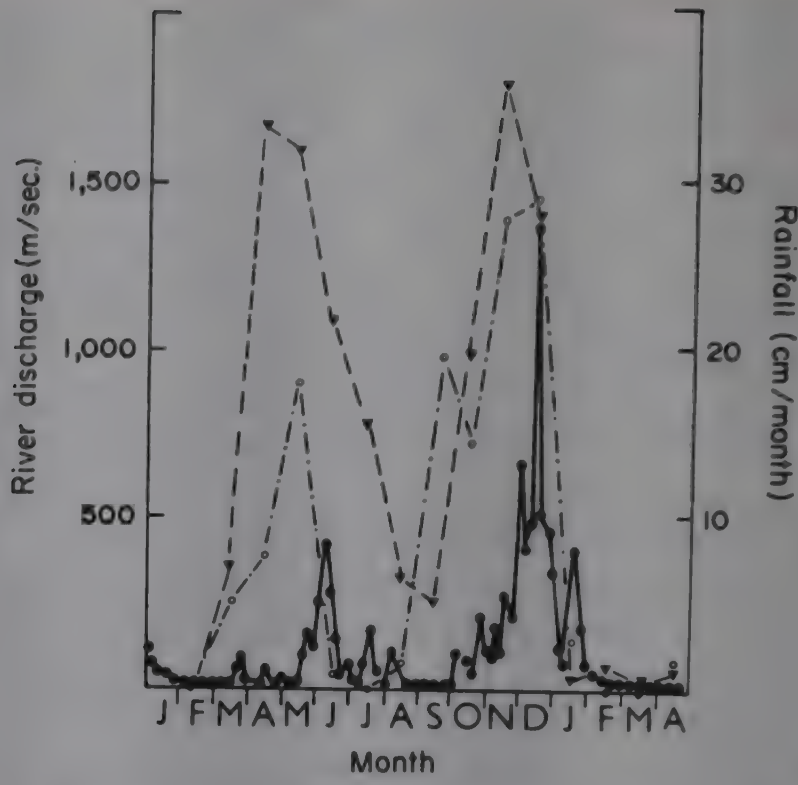

Fig. 2. The monthly rainfall at Katugastota (– – – –) and Polonnaruwa (o — . — . — . o) gauging station (Dept. of Meteorology) and the daily river discharge (o ———— o; mean of data for an 8-day period) at Mannampitya bridge (Dept. of Irrigation) from January 1982 to April 1983.

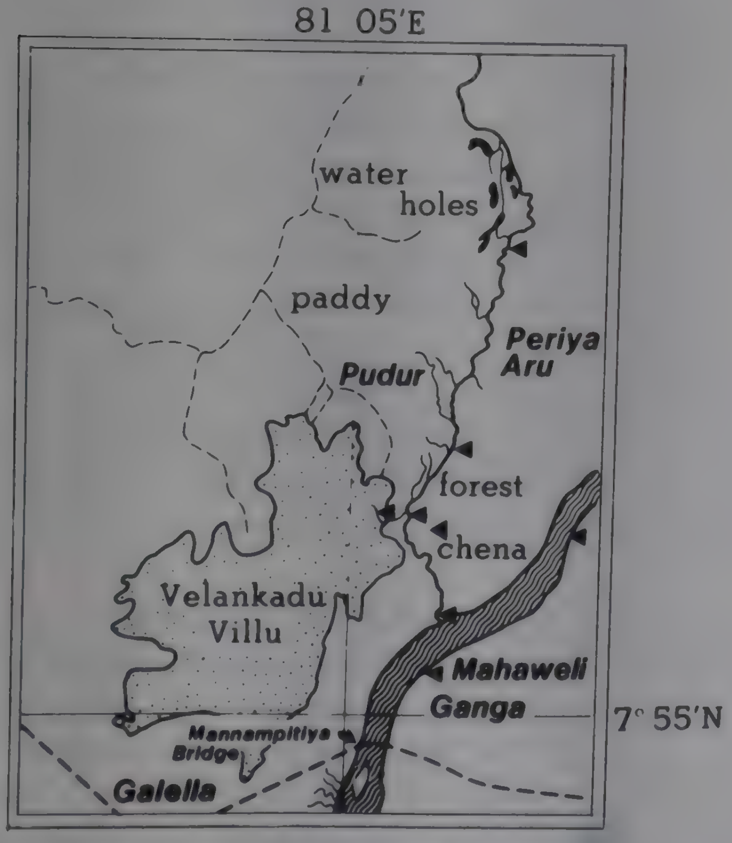
81 05'E
water holes
paddy
Pudur
Periya Aru
forest
chena
Velankadu Villu
Mahaweli Ganga
7° 55'N
Mannampitiya Bridge
Galella

Fig. 1b. Detail of the site. The Periya Aru branching from the Mahaweli Ganga. Velankadu Villu, the major marshland draining into the Periya Aru. Gill net sites.

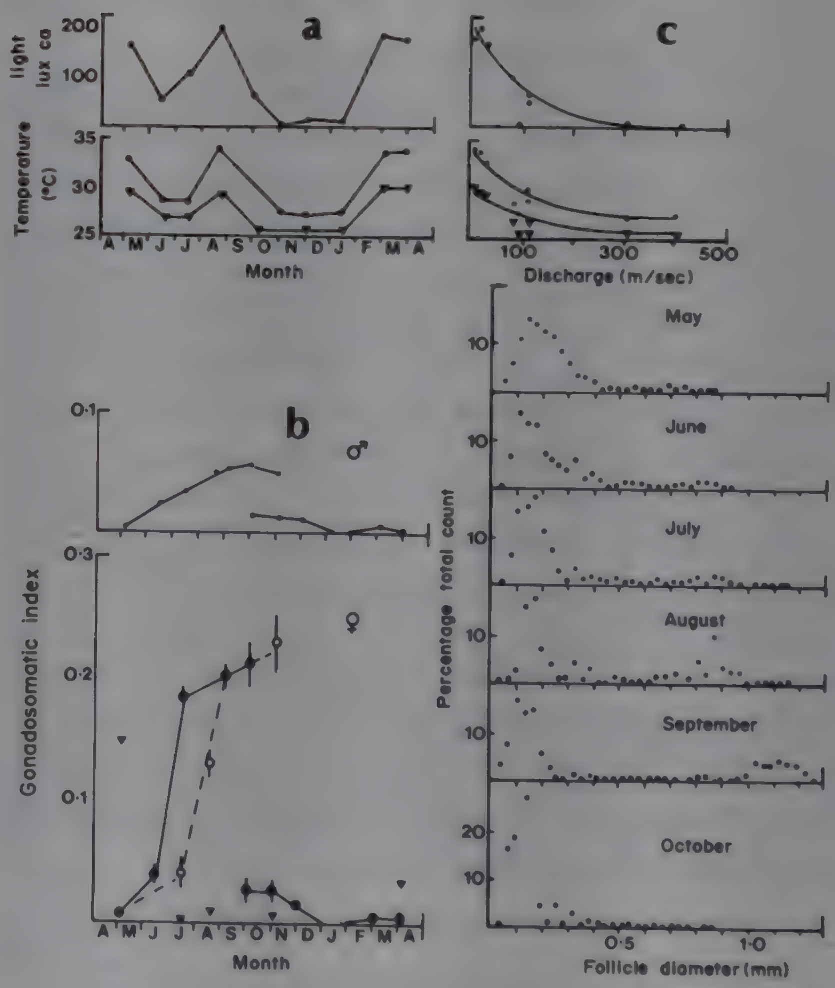

Fig. 3. (a) Change in light intensity and maximum (o ———— o) and minimum (————) temperatures of river water of the Mahaweli Ganga with season and river discharge rate. (b) Change of gonadosomatic index by season in male and female *L. dussemerii*. (c) Egg frequency distribution in the ovary of *L. dussemerii* at various times of the year.

Induced Maturation in Ablated *Penaeus notialis* and *Penaeus schmitti*

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Abstract

Wild *Penaeus notialis* and *P. schmitti* were induced to mature by unilateral eyestalk ablation of females in 0.5, 1.6 and 2.0-m^3 tanks stocked at 9-12 shrimps/m^3 at a ratio of 2♀:1♂. A total of 71 spawns was obtained from 80 *P. notialis* females in two experiments and 29 spawns from 72 *P. schmitti* females in three experiments over 16-19 days per experiment. Average fecundity and hatch rate were 44,410-64,997 eggs/spawn and 0-71.70% for *P. notialis* and 41,672-48,083 eggs/spawn and 0-0.17% for *P. schmitti*, respectively.

The low hatch rates throughout the experiment period for *P. schmitti*, an open thelycum species, and towards the second week for the closed thelycum *P. notialis*, indicate a failure of spermatophore transfer in the maturation tanks. This lack of copulation may be traced to the high stocking densities and the small sizes of the tanks. Attempts to artificially transfer spermatophores to mature *P. schmitti* females were generally unsuccessful.

Introduction

The pink shrimp, camaron rosado *(Penaeus (Farfantepenaeus) notialis)* and the white shrimp, camaron blanco *(Penaeus (Litopenaeus) schmitti)* are the two most important commercial shrimp species in Cuba and the Greater Antilles and along the Atlantic coast of Central and South America (Holthuis 1980). Before 1936, *P. schmitti* was not distinguished from *P. setiferus;* neither was *P. notialis* differentiated from *P. duorarum* until 1967 (Holthuis 1980). Both *P. setiferus* and *P. duorarum* are of great economic importance in Mexico and the United States.

In Cuba, the Ministry of Fisheries has given priority to the culture of *P. notialis* and *P. schmitti.* Recent trials in earthen ponds in Sancti Spiritus province have yielded 800-1,200 kg/ha/year of *P. schmitti,* sizes 14-20 g (M. Borrero, pers. comm). Similarly, trials in research ponds and private farms in Colombia have produced 700-1,400 kg/ha/year (average sizes 15-25 g) in four to six months of culture (Martinez et al. 1984; E. Garcia, pers. comm.).

These promising results have led to further experiments in maturation and seed production. Ramos and Gonzales (1983) obtained maturation and spawning of viable eggs from wild *P. notialis* by eyestalk ablation. Scelzo and Hernandez (1983) induced maturation, but not spawning, by ablation of pond-reared *P. notialis.* So far, 23 penaeid species have been matured in captivity by ablation of one or both eyestalks (Primavera 1985).

This study aimed (a) to induce maturation in *P. schmitti* by ablation and (b) to undertake followup maturation work on *P. notialis.*

Materials and Methods

Adult *P. notialis* and *P. schmitti* were obtained in May and June from Cienfuegos Bay (22°40'N and 80°28'W) using a commercial shrimp trawler (net body and codend stretched mesh 25 mm). Trawling was done in the morning at a velocity of two knots with each operation lasting one hour. Undamaged and healthy shrimp were selected and transported to the Centro de Investigaciones Marinas in Habana in 100-l plastic tanks filled with clean seawater and oxygen.

After sufficient acclimation, immature females and apparently mature males were stocked in tanks of 0.5, 1.6 and 2.0-m^3 capacity at densities of 9-21/m^2 and a sex ratio of 2♀:1♂ (Table 1). Mean CL for P. notialis was 3.2-3.9 mm females, 2.4-2.8 mm males and for *P. schmitti:* 3.6-3.9 mm females, 3.5-3.6 mm males. Females were ablated by pinching one eyestalk as described by Primavera (1978).

Feeding of squid and earthworm at approximately 5% of total biomass was twice a day in the morning and afternoon. Tanks were provided with airstones. Depending on water quality, 20-80% of tank water was changed daily. Temperature, salinity, pH and molts were recorded daily (Table 4). Inclusive experiment dates are: Experiment 1 -

27 May to 12 June1984; Experiment 2 - 25 June to 12 July 1984; Experiment 3 - 24 September to 8 October 1984.

Females were examined for ovarian maturation (with an underwater light or by scooping out the females and holding them against the light) three days after ablation and every day thereafter. Maturation stages were classified according to color (Simpson 1974) and outline or shape of ovaries as described for *P. monodon* in five stages (Primavera 1983).

Stages 3 and 4 females were individually placed in 20-l spawning tanks (54 x 30 x 20 cm) with filtered seawater treated with 1 ppm EDTA. The following morning, spent and unspawned females were returned to their respective maturation tanks. Spent *P. notialis* were marked with Sphirion tags (used for spiny lobster) injected into the abdominal musculature by means of a hypodermic needle to trace possible rematurations.

Eggs were counted in 16 5-ml samples with the Bogorov pipette taken after the water was stirred to obtain a uniform suspension. Nauplii counts were done the same way and hatch rates were computed.

Artificial spermatophore transfer was tried on mature *P. schmitti* that appeared unmated. The sperm was dissected from the spermatophores of apparently mature males and placed together with the glutinous material (Perez Farfante 1974) between the gonopores and the coxal plate of the third pair of pereopods (Bray et al. 1984).

Results and Discussion

Maturation and spawnings were obtained as quickly as two and three days after ablation for *P. schmitti* and *P. notialis*, respectively (Figs. 1 and 2). This is the first recorded captive maturation of *P. schmitti*. Spawns were scattered throughout the two-week period with no discernible peaks for either species. Ramos and Gonzales (1983) also obtained spawnings of wild *P. notialis* at a minimum of three days after ablation.

Almost all of the maturing and mature *P. schmitti* had ovaries with a constriction in the anterior abdominal lobes similar to that described for *P. vannamei* and *P. stylirostris* (Aquacop 1979). The majority (65.5%) of *P. schmitti* spawns were partial, in contrast to the mostly complete spawns of *P. notialis* (Tables 2 and 3). Spawning behavior of *P. schmitti* may require water depth greater than the 15 cm in the spawning tanks. In more recent trials, complete spawns of *P. schmitti* were observed when 100-l plastic containers filled with 30-35 cm (10-l volume) were used (Primavera and Ramos, unpublished data). Size of females in relation to spawning tank size may be discounted as a cause of partial spawns because *P. notialis* females were larger (3.6-4.8 cm average CL) than *P. schmitti* (3.1-3.9 cm average CL) (Tables 2 and 3).

Average egg numbers and hatch rates of spawns from *P. notialis* were 44,410-66,520 eggs/spawn and 0-71.70%, respectively (Table 2). Ramos and Gonzales (1983) reported a range of 13,200-73,200 eggs/spawn with hatch rates of 39.0-86.56% for six wild ablated *P. notialis* with 24-59 g body weight. Martinez et al. (unpublished data) obtained an average of 75,000 eggs/spawn and 93.3% hatch rate from six wild *P. notialis* spawners weighing an average of 55 g.

There is a general trend towards decreased hatch rates both with time (Fig. 3) and with successive rematurations (Table 4) for *P. notialis*. This could be related to a failure of mating or spermatophore transfer in the tanks after molting of the females. In *P. notialis* and other closed thelycum penaeids, spermatophore transfer takes place immediately after molting of the female when it is soft-shelled.

The small size of the tanks (0.7 to 2.0 m^3) and not the stocking density (9-21/m^2) or sex ratio (2♀:1♂) *per se* may explain the lack of copulation. Primavera (1979) observed that the elaborate vertical and horizontal swimming movements in precopulatory and copulatory behavior necessitate a minimum area and depth of tank for *P. monodon*, another closed thelycum species.

Average egg numbers of 41,672-48,083 eggs/spawn for ablated *P. schmitti* (Table 3) are lower than the average of 62,200 eggs/spawn for two wild spawners reported by Martinez et al. (unpublished data). The lack of spermatophores in all mature females suggests that *P. schmitti* did not mate in the tanks, as with *P. notialis*, with the small tank size the most important factor. (In *P. schmitti* and other open thelycum species, maturation and imminent spawning of the female are prerequisite to spermatophore transfer.)

Attempts to artificially transfer sparmatophores to ripe *P. schmitti* females were generally unsuccessful (Table 3). Although this techique has been successfully used for *P. vannamei*, *P. stylirostris*, *P. setiferus* and other penaeids with open thelyca (Aquacop 1979; Bray et al. 1984), application to other species needs some refinement. The exact position of the sperm mass on the thelycum needs to be determined as well as optimum time for transfer of the spermatophores.

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Table 1. Stocking of *Penaeus notialis* and *P. schmitti* in maturation tanks

Tank			A	B	C	D
Shape			rectangular	round	rectangular	rectangular
Area (m^2)			4.0	1.6	0.7	1.0
Water volume (m^3)			2.0	1.6	0.5	0.5
Species:	Expt. 1		*P. schmitti*	*P. notialis*	*P. notialis*	*P. notiala*
	Expt. 2		*P. schmitti*	*P. notialis*	*P. notialis*	*P. notiala*
	Expt. 3		*P. schmitti*	*P. schmitti*		
No. of stock/tank			36	30	15	16
Density (no./m^2)			9.0	18.8	21.4	16.0
Survival (%)						
	Expt. 1	♀	64.2	80.0	40.0	80.0
		♂	87.5	80.0	80.0	20.0
	Expt. 2	♀	46.8	65.0	100.0	80.0
		♂	100.0	100.0	80.0	100.0
	Expt. 3	♀	75.0	88.3		
		♂	83.3	100.0		

Table 2. Spawning of wild ablated *Penaeus notialis.*

Tank	B		C		D	
Experiment no.	1	2	1	2	1	2
Total no. spawns	20	25	7	2	4	11
1st	19	18	7	1	4	9
2nd	1	6	–	1	–	2
3rd	–	1	–	–	–	
Kind of spawning						
Partial	4	10	3	0	1	4
Complete	16	15	4	2	3	7
Ave. carapace length (mm)	3.9	3.8	3.6	4.0	4.8	3.6
Ave. no. eggs/spawn	44,410	64,997	45,910	66,520	54,750	48,091
Total no. eggs	888,200	1,624,917	321,367	132,500	219,000	529,000
Ave. no. nauplii/spawn	17,537	8,420	15,500	47,500	22,438	0
Total no. nauplii	350,750	210,500	108,500	95,000	89,750	0
Ave. hatch rate (%)	39.48	12.96	33.76	71.70	40.98	0

Table 3. Spawning of wild ablated *Penaeus schmitti.*

Experiment no. (tank)	1 (A)	2 (A)	3 (A, B)
Total no. spawns*	6	12	11
Kind of spawning			
Partial	6	10	3
Complete	0	2	8
Ave. carapace length (mm)	3.7	3.9	3.1
Ave. no. eggs/spawn	48,083	41,672	44,304
Total no. eggs	288,500	500,067	487,350
Ave. no. nauplii/spawn	83	0	0
Total no. nauplii	500	0	0
Ave. hatch rate (%)	0.17	0	0

*1st, 2nd and 3rd spawns not differentiated

Table 4. Mean water temperature, salinity and pH at 9 to 10 a.m. in maturation tanks of *Penaeus notialis* and *P. schmitti* (Expt. 2: 26 June-12 July 1984, Expt. 3: 24 September-8 October).

Tank	A	B	C	D
Temperature (°C)				
Experiment 2	27.6 (26.0 – 28.2)	27.6 (26.0 – 28.3)	27.8 (27.5 – 28.0)	27.6 (26.2 – 28.4)
Experiment 3	26.1 (22.5 – 28.0)	26.1 (22.4 – 28.1)		
Salinity (ppt)				
Experiment 2	35.0	35.0	35	35
Experiment 3	35.7	35.7		
pH				
Experiment 2	7.86	7.86	7.87	7.63
Experiment 3	7.82	7.82		

Note No readings were taken for Experiment 1

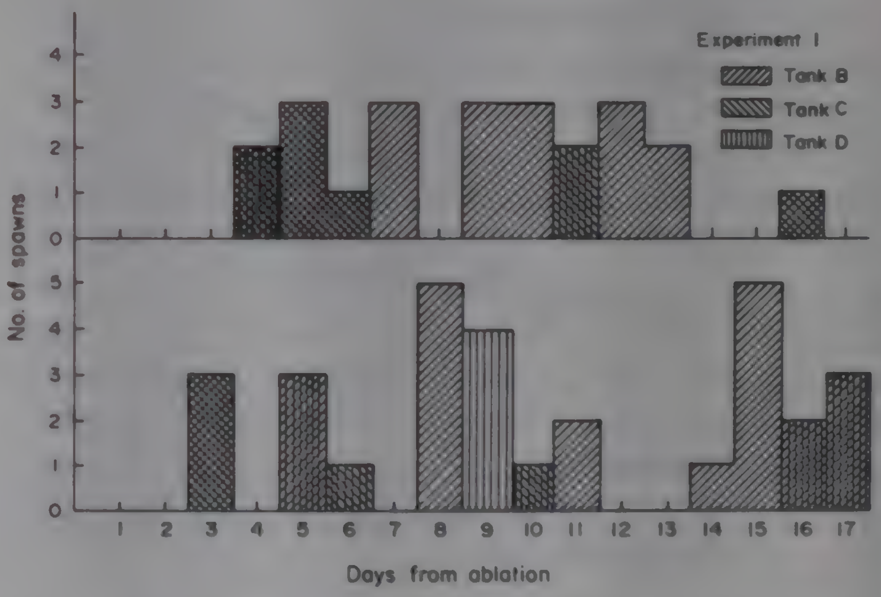

Fig. 1. Spawnings of ablated *Penaeus notialis* in maturation tanks.

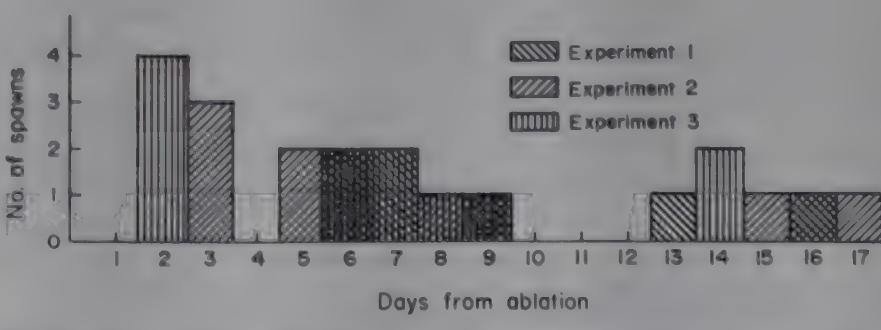

Fig. 2. Spawnings of ablated *Penaeus schmitti* in maturation tanks.

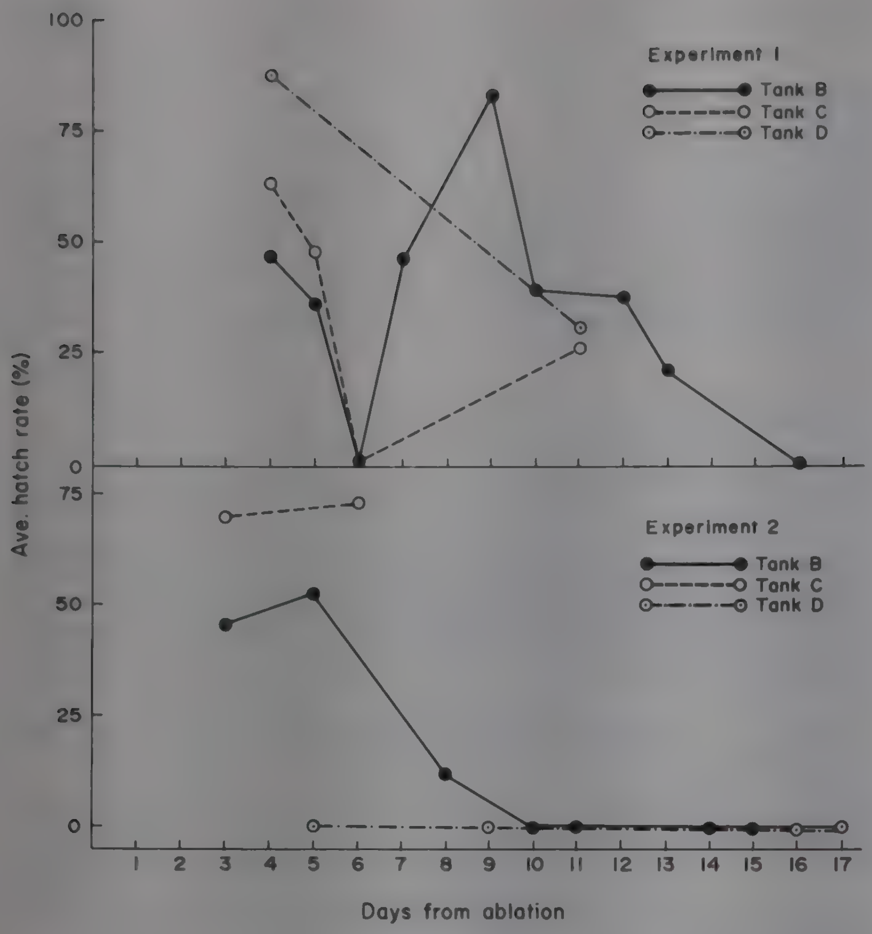

Fig. 3. Hatch rates of spawnings from ablated *Penaeus notialis.*

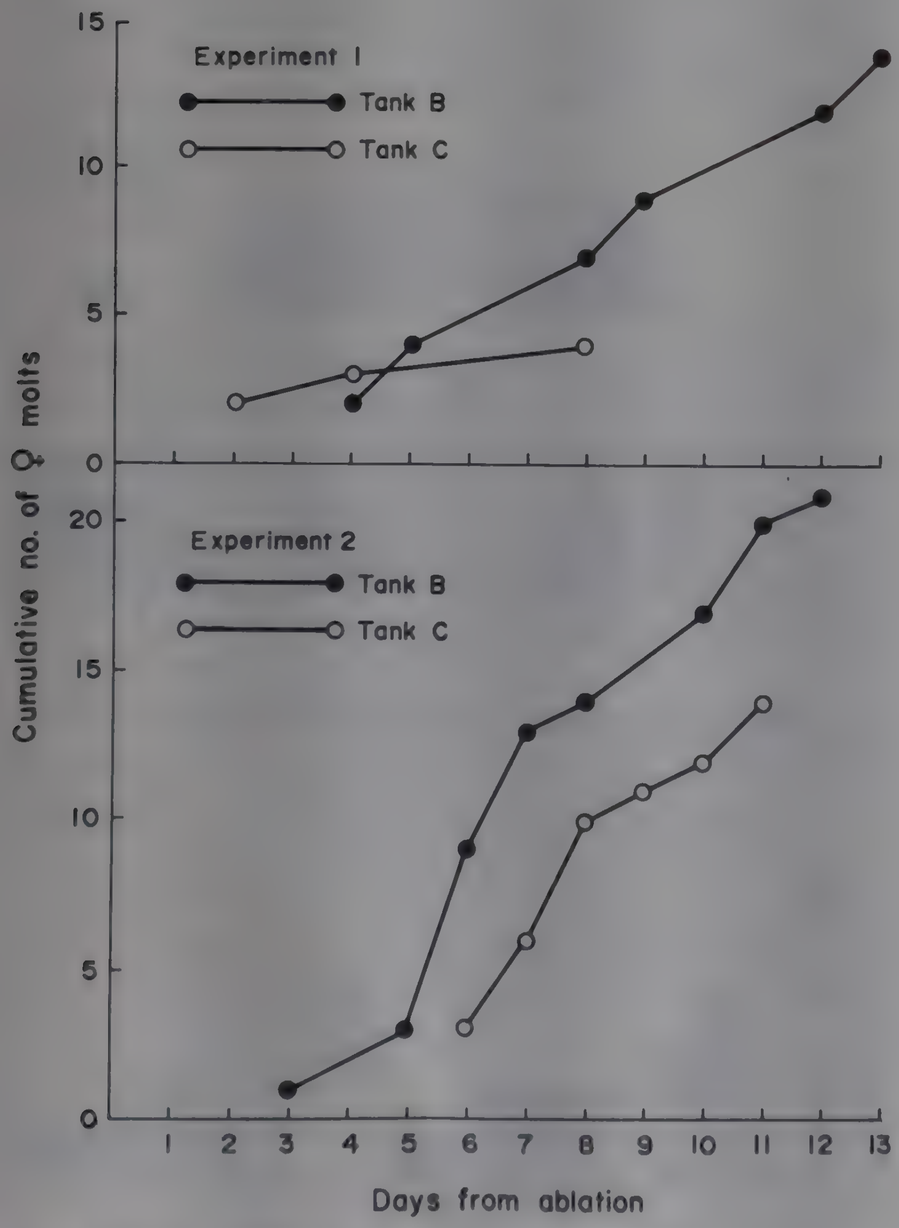

Fig. 4. Molts of *Penaeus notialis* in maturation tanks.

Spawner Size and the Biological Components of the Reproduction Process in *Penaeus monodon* Fabricius

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Abstract

The biological components of the reproduction process of *Penaeus monodon* spawners collected from the wild along Tigbauan-Guimbal coastal area, Philippines, were evaluated. Data were collected on wet body weight, eggs per female, egg size, egg quality, hatching rate and percentage survival at the first zoea stage. There was a general trend for number of eggs per female and egg size to increase with increasing body weight. However, the larger spawners were not the most efficient in terms of number of eggs per unit body weight. There was a significant positive correlation between body weight and number of eggs per female. Larger eggs may be associated with larger body size. However, the size of individual eggs tends to be dependent upon the total number of eggs spawned per female.

Introduction

A species may produce a large number of eggs as in cod, *Gadus morhua* L. or fewer larger eggs, as in freshwater fishes (Blaxter 1969) and some crustaceans (Shakuntala 1977), from which more viable individuals may be hatched to ensure a greater number of surviving juveniles. The success of crustaceans can be attributed to the fact that most of them carry their developing eggs until hatching. This parental care results in greater survival of the eggs. Among the many factors that regulate the number of eggs carried by a female, the size of the mother animal appears to be an important one, e.g., in fishes (Blaxter 1969).

Jensen (1958) working on several marine Matacostraca concluded that the "relative number" of eggs (total number of eggs carried in a single brood at any one time) exhibits a linear relationship to the volume of the mother. This paper reports on observations on the biological components of the reproduction process in relation to the spawner size in *P. monodon* Fabricius.

Materials and Methods

Females of *P. monodon* in apparently good physical condition and estimated to have ripe "Stage IV" ovarian lobes were brought from the catch of small fish farmers trawling along the Tigbauan-Guimbal coastal area. The shrimp bought from several collections from June 1985 to May 1986 were allowed to spawn separately in 250-l conical fiberglass tanks in aerated seawater at ambient temperature (26 ± 2°C). The postspawning weight of each female was taken; number of eggs spawned per female was estimated by taking four 200-ml water samples in which the numbers of eggs were counted. Egg size and egg quality measurements were made under the binocular microscope on 100 egg samples per female. Egg type or quality was classified according to Primavera and Posadas (1981); A_1 and A_2 type eggs were considered good eggs and expressed in per cent. Hatched nauplii were counted as having come from fertilized ova and transferred into 1.5-t fiberglass tanks at a stocking rate of 100,000 nauplii per tonne of seawater. About 20-30% of the water was changed daily by siphoning out detritus to maintain good water quality. There was no feeding until the larvae metamorphosed to the first zoea stage. Survival was estimated by taking a 3-l water sample per tonne of seawater. The water was agitated vigorously during sampling to disperse the larvae evenly.

Results and Discussion

There was a general trend for number of eggs per female and egg size to increase with increasing body weight (Table 1). In contrast, there was marked decrease in the number of eggs produced per unit body weight as body weight increased. Larger eggs may be associated with large body weight. However, the size of the individual egg tended to be dependent upon the total number of eggs spawned per female rather than upon the size of the female.

In many crustacean species, the eyestalk ganglionic X organs or sinus glands are known to contain an "ovarian inhibiting" neurohormone which prevents vitellogenesis. The production of this factor is known to be apparently regulated by environmental stimuli that impinge on sensory receptors. Vitellogenesis commences when the level of this neurohormone is reduced or is absent. The size of each egg then depends on the duration of vitellogenesis during which the yolk is deposited, as well as the total number of eggs competing to share this yolk deposition.

Such observations were quantitatively summarized by correlation analysis (Table 2). There was a significant correlation between spawner size (body weight) and number of eggs per female with a correlation coefficient of 0.66. Significant positive correlations were also obtained between body weight and percentage survival at the first zoea stage and between number of eggs per female and survival at first zoea stage. There was also a definite relationship between egg size and hatching rate and survival of first zoea stage. On the other hand, the negative correlation between egg quality and hatching rate was probably due to the inclusion as good eggs of A_2 type eggs with delayed development and hatching.

Size selection of spawners for maximum egg and larval production is important. In this study, larger females produced more eggs than did smaller females. Similar trends have been recorded for *P. monodon* in captivity by Aquacop (1977) and Motoh (1981) on wild-caught *P. monodon* spawners of 53.1-81.3 mm carapace length. The positive body weight-eggs per female relationship also means that larger spawners will produce a greater number of healthy larvae as indicated by a significant positive correlation between body weight and percentage survival at the first zoea stage (r = 0.42).

Table 1. Body weights and spawning efficiencies of wild *P. monodon* spawners from Tigbauan-Guimbal coastal area

Spawner no.	Body weight (g)	No. of eggs per female	Eggs per unit body weight	Egg size (μ)	Egg quality (%)	Hatching rate (%)	Survival at $zoea_1$ (%)
1	116.3	498,700	4,288	306.4	92.0	84.9	85.1
2	105.6	526,750	4,988	280.1	80.0	53.6	68.6
3	158.7	776,500	4,892	301.0	76.6	88.4	96.8
4	96.1	469,200	4,882	302.2	68.0	86.8	85.0
5	134.6	808,825	6,004	305.7	84.0	92.8	86.8
6	121.3	224,500	1,850	296.0	52.0	98.7	74.4
7	144.2	538,300	3,733	286.4	44.0	58.6	50.7
8	169.7	585,333	3,449	299.8	58.0	85.0	88.7
9	140.6	266,600	1,896	288.4	52.0	46.3	38.9
10	128.8	424,750	3,297	294.5	81.2	49.6	81.9
11	166.4	523,500	3,146	317.2	80.0	65.9	100.0
12	198.7	562,000	2,828	293.0	60.0	60.8	84.4
13	164.8	562,700	3,414	302.6	76.0	64.5	100.0
14	141.8	410,000	2,891	293.0	78.0	70.3	100.0
15	204.8	830,500	4,055	289.1	80.0	51.8	100.0
16	218.6	850,000	3,888	309.3	50.0	74.1	87.8
17	210.4	911,800	4,333	279.7	84.0	60.2	91.8
18	106.5	466,000	4,375	277.4	92.2	56.9	32.1
$\bar{x}$	151.6	568,632	3,789	296.6	69.3	66.7	79.5
S.D.	36.7	190,326	1,046	10.6	14.3	17.7	21.0

Table 2. Correlation coefficients between body weight and the biological components of the reproduction process for wild *P. monodon* spawners.

	Body weight	Eggs per female	Eggs per unit body weight	Egg size	Egg quality	Hatching rate
Body weight	–					
Eggs per female	.66	–				
Eggs per unit body weight	–.15	.63	–			
Egg size	.12	.09	.02	–		
Egg quality	–.14	.19	.40	–.12	–	
Hatching rate	–.25	–.02	.20	.56	–.14	–
Survival at $zoea_1$	.42	.46	.17	.52	.26	.23

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Induced Spawning and Larval Development of the Razor Clam *Solen brevis* Gray (Mollusca: Solenidae) in the Laboratory

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Abstract

The response of gravid razor clams to various physical and chemical stimuli was studied. Gravid razor clams could be induced to spawn by exposure to temperature shock as well as high feed concentrations exceeding 1.1 million cells/ml.

Mature eggs measured approximately 75 μm in diameter and cell division began within 20 minutes after fertilization. The eggs developed into actively moving trochophores within 6 hours and D-stage veligers could be seen after 12 hours. Reared on a diet of *Chaetoceros* sp. the veliger larvae reached the umbo stage within three days and settlement occurred after eight days when mean shell length measured approximately 350 μm. Metamorphosis into the juvenile form was completed after 16-18 days.

Introduction

The razor clams (Family Solenidae) include numerous species exploited commercially as a food resource. Substantial fisheries exist in many countries in the Asia-Pacific Basin. Along the west coast of the United States *Siliqua patula* form the basis of an economically important commercial and recreational fishery throughout its distribution range (Bourne 1979) while *Ensis directus* occupies a similar position on the eastern seaboard. Various species of *Solen* and *Pharella* are commonly marketed in the Southeast Asian countries while *Sinonovacula constricta* has been extensively cultured in Japan and China since the 1900s (Cahn 1951; Nie 1982).

Solen brevis Gray occurs in numerous localities on the west coast of Peninsular Malaysia and Sarawak where they constitute important local fisheries. Their popularity as a food item is such that they command a market value 10 times that of the blood cockle *(Anadara granosa)*. In recent years increasing interest has been shown in the culture of *S. brevis* but efforts towards their mass culture have been hampered by lack of seed supply. Despite its economic importance, little is known about the biology of *S. brevis* and no published information on its spawning and larval biology exist.

Numerous techniques have been used to induce spawning in bivalves (Loosanoff and Davis 1963; Ino 1972; Sastry 1979) and some of these have been successfully used to induce spawning in razor clams (Loosanoff and Davis 1963). More recently, Breeze and Robinson (1981) reported that *Siliqua patula* spawned when exposed to *Pseudoisochrysis paradoxa* at cell concentrations of 2-2.5 million cells/ml.

This paper reports on the induced spawning and larval development of *Solen brevis* in the laboratory.

Materials and Methods

Preliminary studies have shown that the main spawning season for *S. brevis* in Penang is in November-March. Specimens were collected at low tide from the intertidal foreshore off Sungei Nibong, Penang, Malaysia. The clams were immediately transferred to the laboratory and held in running seawater at 32 ppt salinity in shallow fiberglass troughs. Fine sand (5 to 7 cm deep) was provided as substratum. Animals unable to burrow within 30 min. were discarded as their survival was invariably poor. Clams were acclimatized to laboratory conditions for three to five days during which period they were fed a mixture of *Chaetoceros calcitrans* and *Tetraselmis* sp. prior to experimentation. Laboratory temperature varied from 26 to 28.5°C during the experiment.

Prior to experimentation, ten clams from each batch were dissected and their gonads examined microscopically to ascertain that at least 30% were in reproductive readiness (i.e., with motile sperms and mature oocytes). Spawning trials were conducted in 2-l glass bottles, each with six animals. Each treatment was duplicated and repeated on a different day. At the end of an experiment where no spawning was observed, all surviving animals were dissected and examined to ascertain that the absence of spawning was not due to their lack of reproductive readiness. Unless otherwise specified, no feed was provided during the experiments which were conducted at room temperature (26-28°C). Aeration was provided via air diffusers but no substratum was provided. After exposure to each treatment, all test animals were returned to fresh seawater and observed for 48 hours or until spawning occurred. A fresh change of seawater was carried out after 24 hours.

Test temperatures were maintained at preset values through cooling coils and temperature baths (Grants Instruments). Preliminary observations indicated that high mortality occurred when clams were kept at temperature below 20°C or above 35°C for more than a few hours. Test temperatures used for the cold "shock" and warm "shock" were 22-23°C and 32-33°C, respectively. Clams were transferred directly from room temperature to each test temperature, held for three hours and then returned to fresh seawater at room temperature for subsequent observation.

Test animals were immersed in seawater diluted with freshwater to provide final salinities of 15, 20, 25 ppt with those in 32 ppt acting as control. After a 6-hr exposure, the experimental medium was drained off and replaced with fresh seawater.

Experimental animals were immersed for three hours in seawater with ammonium hydroxide added to provide final pH values of 8.6 and 9.6 after which the test solutions were replaced with fresh seawater (pH 7.8).

The mantle cavity of test animals was injected with 0.2 ml and 0.5 ml of 1M KC1 solutions through the inhalant siphon. After receiving the KCl, each clam was left for 10 min. out of water and then returned to fresh seawater. The controls were injected with 0.2 ml and 0.5 ml of seawater, respectively.

Test animals were immersed in seawater with hydrogen peroxide added to provide final concentrations of 50, 100, 150 and 200 ppm H_2O_2 using the procedure of Morse et al. (1978). After three hours the test solutions were replaced with fresh seawater.

Clams were immersed in seawater with the unicellular algae *C. calcitrans* added to provide final concentrations of 2.3, 1.3, 1.1, 0.9, 0.6 and 0.3 million cells/ml. The control animals received no added feed. Cell concentrations were monitored hourly and feed from a stock solution added to maintain cell concentration at each preset value. After 12 hours the test animals were returned to fresh seawater containing no added feed and subsequent observations carried out for a further 36 hours.

Upon release, eggs were left undisturbed for 15-20 min. to complete fertilization. Fertilized eggs were passed successively through a 210-μm and 125-μm sieve to screen off coarse gonadal debris and then collected in a 41- μm sieve. These eggs were gently washed with 15-μm filtered seawater to eliminate excess sperms, counted and then transferred into 15-l glass culture vessels with tapered sides, at a stocking density of 30 eggs/ml. Gentle aeration was provided with air diffusers.

Larval culture was carried out at room temperature with 15- μm filtered seawater (32 ppt salinity, pH 7.8) at an initial stocking density of 5 larvae/ml. The larvae were fed *C. calcitrans* after 24 hours when the D-stage was reached. Initial feed concentration was 30,000 cells/ml. This was raised progressively as the larvae developed. The culture medium was changed every day, during which samples were taken for microscopic examination and larval count. Larvae to be preserved were first killed by adding two drops of Lugol's solution, then fixed in 5% neutral formalin to which sucrose and sodium tetraborate were added to prevent shell shrinkage.

Results

High mortality was observed in clams exposed to ammoniated seawater at pH 9.6, and hydrogen peroxide above 100 ppm as well as at 15 ppt seawater. No spawning occurred in animals exposed to any of these parameters as well as the KC1 treatments at all concentrations tested.

Exposure to the cold temperature "shock" did not result in any spawning, while among those exposed to the warm temperature "shock", spawning occurred in five out of six tests carried out. Spawning occurred between 30 and 40 hours after the warm temperature treatment.

No spawning was observed in razor clams exposed to food at concentrations of 0.6 million cells/ml or less. At concentrations of 0.9 million cells/ml and above, spawning occurred in nine out of ten tests.

For both elevated temperature and high feed concentration treatments, spawning occurred in the evenings, irrespective of the time each experiment was initiated. Thus, when exposure to high feed concentration was initiated in the early evening, spawning occurred within 1-3 hours while in those initiated during the day, spawning began 9 hours later. The males invariably spawned before the females. Sperms were discharged as a stringy whitish mass. These soon broke up, causing the water to turn milky. Eggs were discharged by the females as a yellowish orange stream. In both sexes spawning discharges occurred via the exhalant siphon and lasted up to 30 minutes.

Mature eggs appeared light yellowish and measured 70-77 μm in diameter. Within minutes of release, the eggs were surrounded by sperms and fertilization occurred soon after. Formation of the first polar body was evident within 10 min. Subsequent cell division occurred rapidly and development through the blastula and gastrula stages were passed over the next few hours. Six hours after spawning the newly-differentiated trochophore larvae exhibited circular rotating movements. By the 9th hour, the shell lines began to appear and the straight-hinged stage (D-veliger) was fully formed 11 hours after fertilization.

Measurements of the early larval stages are summarized in Table 1.

The growth and development stages of *S. brevis* larvae in the laboratory, fed *C. calcitrans*, is summarized in Table 2.

Day 1 veligers had mean shell length (SL) of 111 µm. Early umbo stages began to appear by the end of the 2nd day when mean SL had reached 133 µm while mean shell height (SH) was 103 µm. The umbo stage lasted four days during which the larvae fed vigorously and grew rapidly (with SL increasing by approximately 26 µm/day). Development of the larval foot occurred after six days, marking the transition from the late umbo to pediveliger stage. Mean SL and SH were 237 and 187 µm, respectively, at this stage. Settlement began on day 8 and by day 10 almost all the larvae had settled. A high percentage of the settled spats had by this time lost their velum and crawled about with their extended foot. A few days after settlement, SL began to increase disproportionately faster compared to the SH so that by the days 14-16 the spat assumed the typical elongated razor clam form. When subsequently reared in troughs with 1-2 cm of fine sand as substratum and fed a mixture of brown cells, the juveniles showed rapid growth, with mean shell length reaching 6.4 mm in 58 days and 8.9 mm in 94 days.

Discussion

Results show that gravid razor clam scan be induced to spawn by exposure to elevated temperature as well as high concentrations of *C. calcitrans* above 0.9 million cells/ml. These responses of *S. brevis* resemble those of other razor clams, such as *E. directus* which were induced to spawn by exposure to 25°C after prior conditioning at 13°C (Loosanoff and Davis 1963). Spawning of *S. brevis* in response to elevated temperature fits in with field observations that gonadal development is at its highest during the drier months, November-March, when water temperature of the shallow foreshore increases substantially. In India, *Solen kempi* also showed a spawning peak in October-March when the sea becomes warmer (Rao et al. 1962). On the other hand, *S. constricta* from China appear to spawn in the colder winter months (Nie 1982).

The spawning of *S. brevis* when exposed to high food concentration resembles that of *S. patula*, although spawning in the latter was induced at higher concentrations of 2-2.5 million cells/ml of *P. paradoxa* and *Thallasiosira pseudonana* (Breeze and Robinson 1981).

The absence of spawning in *S. brevis* when exposed to chemical stimulation agrees with observations on *S. patula* (Breeze and Robinson 1981). Hydrogen peroxide has been reported to induce spawning in a number of bivalves and gastropods (Morse et al. 1978; Beckvar 1981) while potassium chloride and ammoniated seawater are standard techniques used in the induced spawning of numerous bivalves (Ino 1972). It appears that the Solenidae do not show spawning responses to such direct chemical stimulations.

The size of eggs in *S. brevis* (70-77 µm) is similar to those reported for *E. directus* (Loosanoff and Davis 1963) and *E. siliqua* (Lebour 1938) but smaller than the 90 µm for *S. patula* (Breeze and Robinson 1981). Early embryonic development in *S. brevis* appears considerably faster than in *E. directus* which takes all 11-27 hours to reach the trochophore and D-veliger stages, respectively, when cultured at 27-30°C (Loosanoff and Davis 1963).

The duration of the planktonic larval phase in *S. brevis* (8-10 days) resembles that of *E. directus* (Loosanoff and Davis 1963) and *S. constricta* (Nie 1982) but considerably less than the 20-25 days of *S. patula*. Part of this difference may be due to the effect of temperature (since Breeze and Robinson's studies were conducted at 16.5°C) or differences in the type and quantity of feed used. The settlement size in *S. brevis* (SL = 319 µm) is similar to that of *S. patula* but almost 1.5 times that of *E. directus*. The mean size of three-month old *S. brevis* was 8.9 mm. This is almost twice that reported for *S. patula* where mean shell lengths of 5.0 mm were achieved during a similar growth period (Breeze and Robinson 1981).

The study has shown the technical feasibility of producing *S. brevis* seeds in the laboratory. With a comparatively short planktonic larval phase of only half that of *Anadara granosa* (Wong and Lim 1985), as well as a more rapid larval and juvenile growth rate, *S. brevis* appears attractive for seed production on a hatchery scale. The subsequent availability of *S. brevis* seeds will undoubtedly provide the impetus for the mariculture of these delectable and highly-prized clams.

Acknowledgements

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Table 1. Timing of the early embryonic development of *Solen brevis* (Temperature: 26-28°C, salinity: 32 ppt).

Stage	Approximate time after fertilization	Egg dia/shell length (μm)
Egg	–	70-77
Appearance of 1st polar body	10 min.	
1st cleavage	20 min.	
2nd cleavage	30 min.	
Trochophore	6 hr	75-85
Early D-shaped	12 hr	96-107

Table 2. Growth and development in *Solen brevis* larvae fed on *C. calcitrans* (Temperature: 26-28°C, salinity: 32 ppt).

Time (hr)	Development stage	Shell length (M ± SD) N = 30 (μm)	Feed conc. (cells/ml)
12	Early D-shaped	102 ± 4	–
1	D-shaped	111 ± 7	30,000
2	Early Umbo	133 ± 8	50,000
4	Umbo	187 ± 12	50,000
6	Late Umbo/Pediveliger	237 ± 34	75,000
8	Settlement	319 ± 53	100,000
16	Metamorphosis	969 ± 53	100,000

Gonadotropin and 17β-Oestradiol Changes During Induced Spawning and Annual Reproductive Cycle in Wuchang Fish *(Megalobrama amblycephala)*

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Abstract

The radioimmunoassay (RIA) for 17β -oestradiol (17β -E_2) of Wuchang fish was developed. A synthetic analog of hypothalamic luteinizing hormone (LRH-A) was used as a stimulant for induced spawning in Wuchang fish *(Megalobrama amblycephala)*. Both serum gonadotropin (GTH) and 17β -E_2 levels were measured by means of RIA. For 17β -E_2 RIA, the accuracy (recovery, 92.81 ± 8.16%), specificity (cross-reactions with oestrone and oestriol, < 3.20 and 2.99%) and sensitivity (minimum measurement, 2.36-2.50 pg) were determined. The standard curve, $r = -0.998$, $s = 0.023$-0.051, the measurement range was from 10 to 400 pg/tube. After injection of LRH-A, serum GTH level rose to about 17 times the prespawning level in fish which spawned, but only 3-5 times in fish which did not spawn. These changes in serum GTH level were similar to those for grass carp and silver carp during induced spawning. The 17β -E_2 level significantly declined after induced spawning with HCG + CPG and LRH-A. In addition, an annual reproductive cycle of 17β -E_2 level was measured. A positive correlation ($r = 0.6089$) was found between serum 17β-E_2 levels and the gonadosomatic index (GSI). The 17β-E_2 levels rising preceded GSI increase. The peak level of 5.63 ± 0.85 ng/ml in April was a month before spawning season. This may be due to quick accumulation of yolk in the oocytes, after which the level decrease. A small peak from October to December may be responsible for oocyte vitellogenesis during the next reproductive cycle.

Introduction

Synthetic LRH-A, and analog of luteinizing hormone releasing hormone (pGlu.His.Try.Ser.Try.A-Ala.Leu.Arg.Pro.NH.C_2H_5) has been applied to carp propagation extensively since the 1970s and extremely significant effects have been obtained (Symposium of Hormone Research 1975; Fukien-Keangsu-Shanghai Cooperative 1976, 1977; Cooperative Team for Hormonal Application 1976). It is an effective inducing agent for fish spawning. In recent years, the action and the inducing spawning mechanism of LRH-A have been studied either on ultramicrostructure of gonadotropic cells in hypophysis or on histological chemistry of hypophysis and ovary (Academia Sinica et. al. 1977a, 1977b; Fang et al. 1981).

With the use of rdioimmunoassay (RIA), the study of changes of serum GTH level in fish during spawning induced by LRH-A was carried out, indicating the contribution of synthesis and release of GTH to the modulation and control of spawning (Jiang et al. 1980; Pau et al. 1980; Xu et al. 1981; Zhao et al. 1981; Lin 1982; Kraak et al. 1983). The RIA this experiment developed recently for measuring the 17β -oestradiol (17β -E_2) in fish serum has laid a foundation for reseach on the function of sexual hormones in fish.

Materials and Methods

Blood samples were taken from fish just around spawning. These fish were collected from fish farms in Xiamen and Shanghai in 1979 to 1984. Blood was removed from the caudal vein of each fish and centrifuged at 3000 rpm for 5 min. The serum was collected and stored at -40°C until assay.

Serum GTH levels of Wuchang fish were measured by RIA with the double antibody method (Fish Reproductive Physiology Research Group 1978), while the 17β -E_2 concentration with the RIA kit produced by Shanghai Institute of Endocrinology (Ding-Ting et al. 1981) and Shanghai Birthcontrol Institute. The method of measurement was slightly improved.

Of the diluted serum 0.5 ml was extracted with 3.0 ml ether and vortex-mixed for 1 min. The aqueous phase was frozen in dry-ice/acetone and the supernatant decanted. Two extracts were evaporated in 40-50°C water bath and 10.0 μl of titrated 17β -E_2 (≈13,000 cpm) was added to each assay tube. After the extracts evaporated in the same way as ether, 0.2 ml of 1:4,000 or 1:15,000 17β- -E_2 antiserum was added to each tube and vortexed. All tubes were incubated at 4°C for 16-24 hr. Then 0.1 ml of 0.5% GPBS was added to each tube. A few min later, 0.5 ml of dextran-coated charcoal suspension (0.25%) was added to each tube and mixed. After standing in an ice bath for 15 min. the samples were centrifuged (3,000 rpm for 10 min. at 4°C) and the supernatants were poured into scintillation vials to which 8.0 ml of scintillation fluid was added. The vials were vortexed for 1 min. and counting was carried out 4 hours after equilibration.

The concentration of 17β -E_2 in serum was calculated according to the established standard curve. The logarithm of 17β -E_2 standard was plotted as a log transformation of B/B_0. For the standard curve (Fig. 1), $r = -0.998$, $s = 0.023$-0.051 (standard deviation of regression), sensitivity was 2.36-2.50 pg and measuring range 10-400 pg/tube.

The RIA procedures used were based on measurements from extracted serum samples. Extraction recovery tests were carried out; the extraction rate of serum sample was 91% (n = 7).

Varying amount of unlabeled 17β-E_2 (50, 100, 150 and 200 pg) were added to serum prior to extraction and the quantities of added 17 -E_2 were compared with measured concentration. The average recovery rate was 92.81 ± 8.61% (n = 15) (Fig. 2).

From Wuchang fish, 0.125, 0.25, 0.50 and 1.00 ml of serum were taken for measuring the linear relationship between the measured absolute concentration of 17β-E_2 in the serum and the amount of the sampling serum. The result essentially showed a straight line passing through the origin of coordinates with the correlation coefficient, $r = 0.9923$ (Fig. 3).

The cross-reaction tests of 17β -E_2 antiserum with six steroids added to Wuchang fish serum were conducted and the binding rates are shown in Table 1.

Results

Female Wuchang fish reared in ponds do not spawn automatically. The GTH concentration in serum of these fish was low (7.71 ± 6.29 ng/ml, n = 15). The GTH level in serum of female Wuchang fish, which spawned in 24 hr after an injection of LRH-A (10-100 μg per kg of fish weight and water temperature, 25°C), increased markedly. It was as high as 133.7 ± 114.29 ng/ml (n = 10) and 13-17 times higher than that of fish before spawning ($p < 0.001$).

The serum GTH concentration in female Wuchang fish which had not spawned after an injection of LRH-A also increased but did not increase as much as those which spawned. It was only 28.40 ± 21.00 ng/ml (n = 5), 3-5 times compared to that before an injection of LRH-A. The GTH levels in serum between spawned fish and unspawned fish after the injection of LRH-A were significantly different ($p < 0.02$) (Fig. 4).

Before spawning, the 17β-E_2 serum levels of two groups of Wuchang fish with their gonads developing beyond the fourth stage were 1,392.71 ± 399.09 pg/ml (n = 6) and 1,219.29 ± 420.51 pg/ml (n = 10), respectively.

The 17β -E_2 levels decreased significantly ($p < 0.001$) in each group after they were injected either with HCG plus dried pituitary gland of carp (CPG) (1,250 I. U. of HCG + 2 mg of CPG/kg of fish) or with LRH-A (10-100 μg/kg of fish). They were 346.71 ± 129.51 pg/ml (n = 4) nd 324.28 ± 228.00 pg/ml (n = 10), respectively (Fig. 5).

Levels of 17β-E_2 in serum of female Wuchang fish were measured during the annual reproductive cycle with RIA: 17β -E_2 level from a minimum, 0.61 ng/ml in January to a maximum, 5.63 ng/ml in April and then declined. In September, the 17β -E_2 level was low (0.74 ng/ml). From October, the level rose slightly in October, November and December to around 1.65 ng/ml. A positive correlation ($r = 0.6089$) was found between 17β -E_2 level and gonadosomatic index (GSI) and 17β-E_2 rising before GSI (Fig. 6). The coefficient of correlation from January to April was $r = 0.8389$ (Fig. 6). The peak level of 17β -E_2 was about a month before the spawning season. The results show that significant change of serum 17β -E_2 in Wuchang fish occurred before spawning especially in early spring.

Discussion

In salmonoids and cyprinid, the serum (or plasma) GTH levels are low before spawning but increases during and after spawning (Crim. et al. 1975; Zhao et al. 1981; Jiang et al. 1980). In our experiments, similar findings were obtained. However, the serum GTH of those non spawners which were injected with LRH-A was low (28.40   21.00 ng/ml). This indicates that the serum (or plasma) GTH levels of salmonids and cyprinids increased during spawning, which can be considered as spawning index for these species.

Differences of GTH level during spawning are found among teleost species, which may be due to species specificity. During spawning, serum GTH concentration was ten or more times higher than the prespawning level. It may modulate and control the process of synthesis and the release of sex hormones and lead to the final maturation of oocytes. No significant difference in serum 17β -E_2 level was observed between fish injected with HCG plus CPG and those injected with LRH-A. This result seems to indicate that the physiological effects of the exogenous gonadotropin on inducing endogenous 17β -E_2 production are the same as those of the endogenous GTH secreted by fish itself.

When the ovary of Wuchang fish is immature, the low level of serum 17β-E_2 may strengthen the activity of monoamine oxidase and inhibit the release of prolactin via catecholamine action. Thus, the activity of adengylate cyclase in the hypophysis can be strengthened and the systhesis and accumulation of GTH in hypophysis may be induced. Whenever the ovary is mature, the high level of serum 17β-E_2 will inhibit the activity of monoamine oxidase (Olcese and de Vlaming 1979). The hypothalamus, however, still release a certain amount of LRH to maintain a low level of serum GTH. Once exogenous LRH or LRH-A has been introduced to the fish, a great amount of GTH which has been synthesized and accumulated in the hypophysis will be released producing a serum (or plasma) GTH peak during spawning time. In immature fish, according to the results of this experiment, 17β-E_2 may act on the hypothalamus-hypophysis axis as a positive feedback to promote the synthesis and accumulation of GTH. A negative feedback of 17β-E_2 may inhibit the hypothalamus-hypophysis axis to release GTH (Bommelaer et al. 1981; Peter 1982). A high level of blood GTH may in turn inhibit 17β-E_2 release from gonad. This mechanism was observed from in vitro study of ovarian follicles incubated with GTH; less 17β-E_2 was produced when the follicles were incubated with a high dosage of GTH (Zhao and Wright, in press).

Therefore, there is an automatic control between the 17β-E_2 and the hypothalamus-hypophysis axis during gonad development and reproduction. In our experiments, changes of serum 17β-E_2 were closely related to the formation and accumulation of yolk. Increasing serum 17β-E_2 levels stimulates the elevation of the precursor yolk protein in female fish plasma during gonad development. This protein is transported to the oocytes by blood circulation and incorporated as yolk (Van Bohemen et al. 1982; Katsumi 1973; Lambert and Bosman 1978). A peak level of 17β-E_2 in April may be a sign of quick accumulation of yolk in oocytes at early stage IV of ovary, but not in relation to gonad development at late stage IV. A small peak in October-December may be responsible for oocyte vitellogenesis during the next reproductive cycle. Thus, it may be possible that when fish are given a certain amount of exogenous 17β-E_2 during the stage of vitellogenesis, the formation of vitellogenin in the liver may be promoted and the gonad development accelerated. This may be an effective means of promotion of gonad development in fish farm.

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Table 1. Percentage cross-reaction of six steroids with 17β-E_2 antiserum.

Steroid	Oestrone	Oestriol	Progesterone	Testosterone	Cortisol	Corticosterone
Binding rate	<3.20	<2.99	<0.01	<0.01	<0.01	<0.01
n	10	8	7	8	7	5

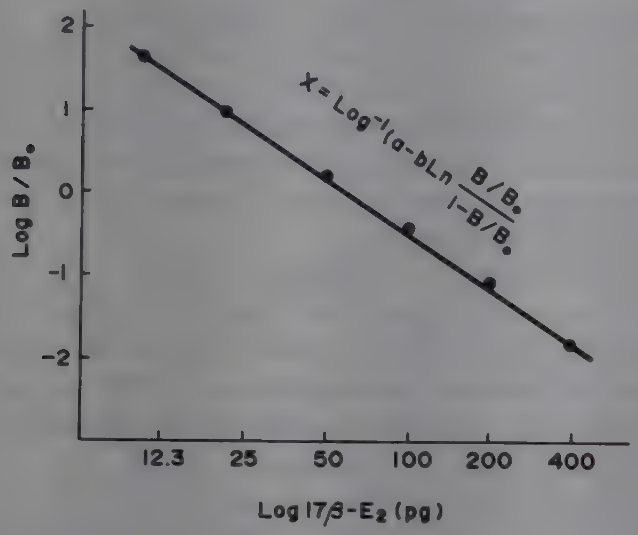

Fig. 1. Logit transformation of standard curve.

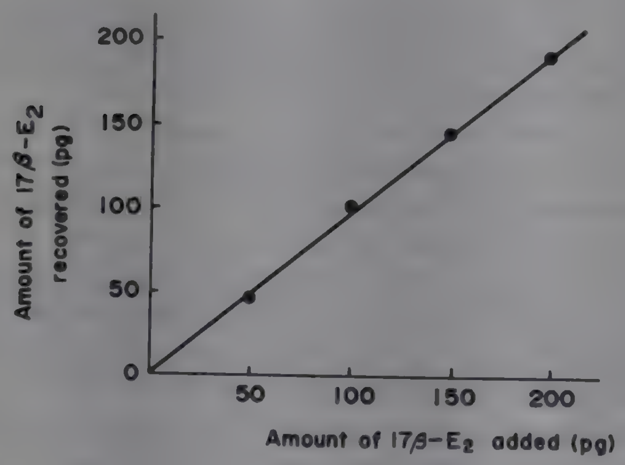

Fig. 2. Recovery test of 17β-E_2.

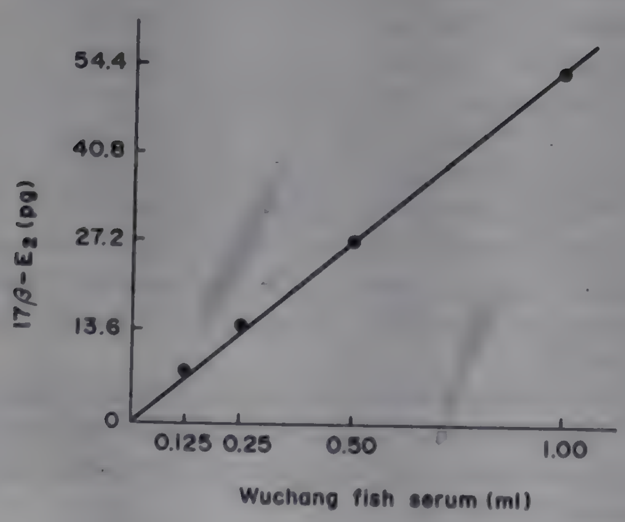

Fig. 3. Dilution test of serum.

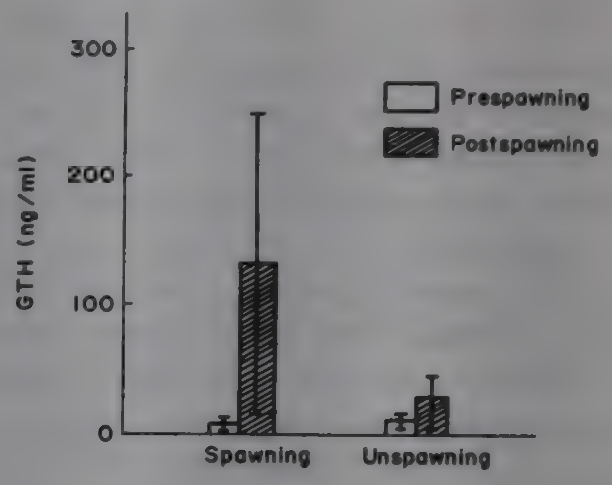

Fig. 4. Changes of serum GTH level in female Wuchang fish during induced spawning with LRH-A.

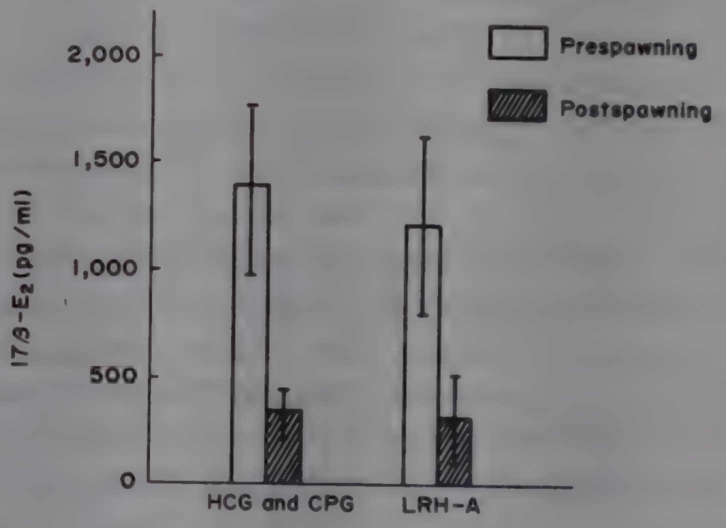

Fig. 5. Changes of serum 17β-E_2 level in female Wuchang fish induced spawning.

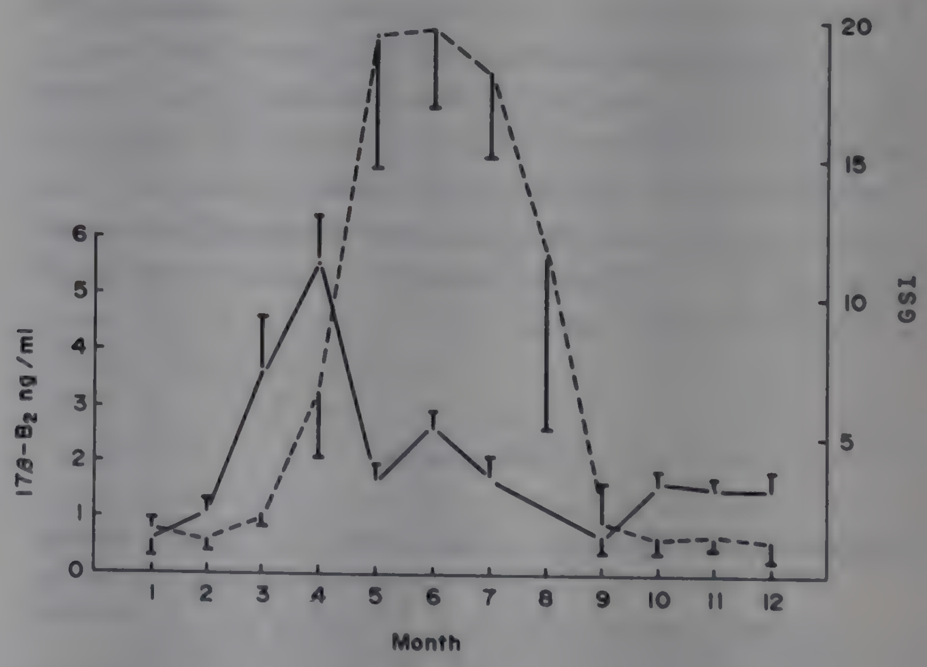

Fig. 6. Serum 17β-E_2 level and GSI changes in Wuchang fish *(Megalobrama amblycephala)* during annual cycle. ——— E_2, ----- GSI.

Species Index

Acanthaster 300
Acanthaster sp. 300
Acanthaster plancii 487, 488
Acanthopagrus cuvieri 434
Acanthopagrus schlegeli 219
Acanthopeltis 165
Acetes 581, 582, 583
Acetes sp. 386, 581
Acetes erythraeus 386
Achirus lineatus 653
Achlya 337, 338
Achlya flagellata 338
Achlya prolifera 338, 339
Achromobacter 244, 245
Achromobacter sp. 456
Acinetobacter 236, 244
Acinetobacter sp. 456
Acinetobacter/Moraxella 438
Acropora 300, 487, 488
Aega sp. 251
Aegathoa 252
Aerococcus viridans (var.) *homari* 331
Aerococcus viridians 332
Aeromonas 224, 225, 235, 237, 243, 244, 245, 337, 338, 438
Aeromonas sp. 241
Aeromonas spp. 464
Aeromonas hydrophila 224, 225, 228, 229, 231, 232, 235, 236, 237, 241, 242, 327, 338, 339, 456
Aeromonas hydrophila biovar *hydrophila* 223, 227, 228
Aeromonas liquefaciens 231, 339
Aeromonas punctata 231, 237
Aeromonas salmonicida 225, 231
Aeromonas sobria 237
Alcaligenes 244
Alcaligenes/Pseudomonas 244
Alexandrium 307
Alitropus typus 263
Allomyces 337
Allomyces sp. 338
Alteromonas sp. 331, 332
Alteromonas putrefaciens 456
Ambliphrya sp. 261, 262
Amblygaster sirm 459
Ammodytes personatus 180
Ampelisca brevicornis 195
Ampeliscidae 193, 194
Amphidinium 308
Amphidinium carterae 307, 308
Amphidinium klebsii 307, 308
Anabaena 609, 610, 611
Anabaena sp. 567, 609
Anabaena azollae 523
Anabus 390
Anacropora puertogalerae 488
Anadara sp. 285, 286
Anadara granosa 703, 705
Anchoa mitchilli 653
Anchoviella indica 459
Anguilla japonica 37, 161, 162, 163, 219, 520
Anisakis sp. 252
Ankistrodesmus sp. 567
Anomura 193, 194
Anthelia 300
Anthocidaris crassispina 361, 362, 363
Apiosoma 256, 257
Apiosoma sp. 255, 261, 262
Archosargus rhomboidalis 653
Argulus 263
Argulus sp. 252
Ariidae 367
Aristichthys nobilis 125, 157, 235, 236, 247, 265, 266, 269, 270, 271, 273, 669, 687
Artemia 212
Artemia sp. 68
Artemia salina 9, 45, 68, 581, 589
Auxis rochei 371, 372
Auxis thazard 371
Avicennia 286
Azolla 523, 524, 525
Azolla pinnata 523, 524, 525
Bacillus 235, 236, 337, 438
Bacillus sp. 338
Bacillus cereus 334
Bacterionema sp. 331, 332
Bagridae 369
Bagrus 274
Bagrus docmac 274
Barbatia paravillosa 312
Barilius 414
Betta splendens 327
Bombyx mori 157
Botia 390
Brachionus 211, 212, 663, 664
Brachionus sp. 212, 581
Brachionus spp. 212

Brachionus plicatilis 9, 68, 114, 180, 619
Brachyotrichia quoyi 290
Brachyura 193, 194, 251
Bucephalus sp. 251, 252, 253
Calanus 569, 570, 571
Caligus 257, 262, 263
Caligus sp. 251, 252
Caligus epidemicus 255, 257, 261, 262
Caligus patulus 263
Callinectes sapidus 46
Camellia sp. 79
Candacia 571
Capitella capitata 194, 195
Capitellidae 193, 194
Carangidae 350, 358
Caranx sp. 467
Caranx tille 372
Carassius auratus 273
Carassius auratus cuvieri 397
Carassius auratus grandoculis 397
Carassius aureatus gibelio 105
Carassius cuvieri 117, 118, 125
Carcharhinus falciformes 372
Carcinus maenas 630
Cardicola sp. 252
Carpilius maculatus 479
Catla catla 88, 414, 565, 603
Centriscus scuttatus 350
Ceratophyllum 689
Cerithidea sp. 285
Cestoda 251
Chaetoceros 42, 581, 582
Chaetoceros sp. 581, 582, 703
Chaetoceros calcitrans 703, 704, 705
Chaetodontidae 386
Channa spp. 414, 415
Channa micropeltes 327
Channa striata 50, 145, 390
Chanos 651
Chanos chanos 49, 63, 75, 89, 90, 141, 223, 227, 255, 261, 262, 263, 519, 543, 547, 574, 653, 671
Chara 556
Chichlasoma meeki 279
Chicoreus ramosus 53, 56
Chilodonella sp. 252
Chlamydomonas sp. 303
Chlorella 180, 185, 619, 620
Chlorella sp. 303, 581
Chlorella spp. 68
Chondrococcus columnaris 225
Chromobacterium 235
Chromobacterium sp. 236, 237, 456
Chromobacterium violaceum 338
Chroococcus dispersus 611
Chrysophrys major 10, 585
Cichlidae 201, 255
Cichlidogyrus 256, 257
Cichlidogyrus spp. 255, 256, 257
Cichlidogyrus branchialis 255
Cichlidogyrus longicornis longicornis 255, 256
Cichlidogyrus sclerosus 255, 256, 257, 266
Cichlidogyrus tiberianus 255, 256
Cichlidogyrus tilapiae 255, 256
Cirrhigaleus barbifer 199
Cirrhina mrigala 88, 414
Cirrhinus jullieni 390
Cirrhinus molitorella 157, 668
Cirriformia tentaculata 194, 195
Citrobacter 145, 235
Citrobacter sp. 237
Citrobacter spp. 464
Citrobacter freundii 236, 237
Cladophora sp. 137
Clarias 390
Clarias batrachus 50, 231, 241, 683, 684
Clarias lazera 669
Clarias macrocephalus 683, 684
Claridae 369
Clausocalanus 571
Clinocottus analis 161, 162, 163
Clostridium botulinum 474
Clostridium perfringens 464, 474
Clostridium sporogenes 474
Clupea 652
Clupea spp. 68
Clupea harengus 169, 652, 653
Clupea pallasi 574
Clupeidae 386
Cochlodinium 307
Cochlodinium sp. 309
Coelastrum sp. 567
Coenobita sp. 479
Colisa lalia 585
Coolia 308
Coolia monotis 308
Copepoda 251, 255, 261, 262
Corica 390
Corica goniognatus 390
Corophiidae 193, 194
Corynebacterium 243
Coryphaena hippurus 371, 372, 463
Cottidae: Scorpaeniformes 161
Crangon crangon 638
Crangon vulgaris 639
Crassostrea 447
Crassostrea angulata 294
Crassostrea belcheri 59, 60
Crassostrea commercialis 429, 430, 438

Crassostrea gigas 294, 315, 316, 317, 348
Crassostrea iredalei 293, 294, 429, 437
Crassostrea madrasensis 59, 60
Crassostrea virginica 320, 429, 430, 438
Crustacea 193, 194, 208, 570
Cryptobia 256, 257, 262
Cryptobia branchialis 255, 256, 257, 261, 262
Cryptocaryon 253
Cryptocaryon sp. 252
Ctenopharyngodon idella 107, 157, 235, 236, 247, 253, 270, 390, 425, 687, 669
Cucullanus sp. 251
Cyanophyceae 89
Cyanophyta 611
Cycloplectanum (= Diplectanum) latesi 251
Cycloplectanum sp. 252
Cycloplectanum spp. 252
Cycloplectanum epinepheli 243
Cycloplectanum latesi 251, 252
Cymatium muricinum 53, 56
Cymodocea 507
Cymothoa sp. 252
Cyprinus sp. 460
Cyprinus carpio 10, 125, 133, 134, 145, 149, 157, 169, 219, 241, 253, 280, 281, 367, 397, 425, 520, 539, 560, 561, 577, 578, 603, 625, 668
Cyprinus carpio var. *communis* 565
Cytophaga 219
Cytophaga sp. 219
Cytophaga/Flexibacter 244
Dactylogyrus 265
Dactylogyrus sp. 251
Dactylogyrus anchoratus 265
Dactylogyrus aristichthys 265
Dactylogyrus extensus 253, 265
Dactylogyrus nobilis 265, 266
Dactylogyrus vastator 253, 265, 266
Dactyloptena orientalis 350, 351
Dactyloptena orientalis (Dactylopteridae) 349
Dactylopteridae 349, 350
Dalatiidae 199
Danio 414
Danoides microlepsis 390
Daphnia carinata 529, 530
Dascyllus trimaculatus 298
Dasyatidae 349, 350
Dasyatis uarnak 350
Dasyatis zugei 350
Daya jerdoni 350, 351
Deania eglantia 199
Decapterus 512
Decapterus spp. 511
Decapterus akaadsi 511, 512
Decapterus macrosoma 443, 511
Dentaliidae 193, 194
Dermogenus pustillus 327
Derris elliptica 413
Derris lagensis 413
Derris uligmosa 413
Diaphus suborbitalis 569, 570, 571
Diaphus watasei 571
Digenea 251, 255, 261, 262
Dinophysis 307
Dinophysis acuminata 308
Dinophysis caudata 308
Dinophysis fortii 307, 308
Dinophysis tripos 308
Diospyros cordifolia 413
Drepane punctata 350
Dunaliella sp. 212
Dussumiera spp. 215
Echinometra sp. 382
Ectenurus sp. 251
Edwardsiella sp. 145, 463
Eichhornia 556, 683
Eichhornia crassipes 577
Elagatis bipinnulatus 371, 372
Eleotridae 367
Eleutheronema tetradactylum 80
Eloda 690
Engraulidae 386
Engraulis 652
Engraulis mordax 654
Enhalus 507
Enhalus acoroides 190
Ensis directus 703, 705
Ensis siliqua 705
Enterobacter 145, 235, 456
Enterobacter sp. 236, 237
Enterobacter aerogenes 463, 464
Enterobacteriaceae 236, 244, 256
Enteromorpha sp. 137
Enteromorpha flexuosa 289, 290
Epinephelus spp. 434
Epinephelus morio 663
Epinephelus salmoides 663, 664
Epinephelus tauvina 663
Epistylis sp. 42, 138, 327
Errantia 194
Erwinia sp. 236
Escherichia coli 11, 145, 146, 236, 293, 294, 429, 447, 448
Etheostoma spectabile 654
Etroplus suratensis 353, 354
Etrumeus teres 512
Euchaeta 570, 571
Eucheuma 54, 55
Euphausia pacifica 180

Euphyllia 488
Euphyllia fimbriata 488
Euthynnus affinis 371
Euxiphipops navarachus 298
Evynnis japonica 113
Extenurus sp. 252
Favia 487, 488
Favites 488
Flavobacterium 235, 237, 244, 245, 337, 438
Flavobacterium sp. 236, 338
Flavobacterium/Cytophaga 244
Flexibacter 219, 220
Flexibacter sp. 219, 220
Flexibacter columnaris 219, 220, 241
Flexibacter marinus 219, 220
Foraminifera 194
Fusarium 337, 338
Gadus 654
Gadus morhua 653, 701
Galaxea 487, 488
Gambierdiscus 307
Gambierdiscus toxicus 307, 308
Gambusia affinis 367
Gasterosteus aculeatus 102
Gastrophysus scleratus 350, 351
Gastropoda 194
Gelidiella 165
Gelidium 165
Gelidium sp. 165, 488
Gelidium filicinum 165
Gelidium lingulatum 165, 166
Gelidium rex 165, 166
Gerridae 418
Gessnerium 307
Glossogobius giurus 79, 80
Glossolepis multisquamatus 368
Glycera lancadivae 194
Gnathiidae gen. sp. 262
Gnathopogon elongatus caerulescens 10
Gobiidae 367
Golfingia sp. 195
Goniadidae 193, 194
Gracilaria 165
Gracilaria crassa 289, 290
Grammatorcynus bilineatus 372
Gymnodinium 307
Gymnodinium sp. 308, 309
Gymnodinium nagasakiense 307, 308
Gyrodactylus 257, 265
Gyrodinium aureolum 309
Haematodinium 331
Hafnia alvei 464
Halimeda opuntia 488
Halodule
Halodule pinifilia 190
Halophila 507
Halophila ovalis 190
Hampala macrolepidota 515, 516, 517
Haplochromis 274
Haplorchus okogawai 263
Helostoma temmincki 263, 273, 530
Homarus americanus 46, 589, 590, 615, 616, 658
Homarus gammarus 46
Hydrilla 689
Hypnea cornuta 289, 290
Hypophthalmichthys molitrix 125, 157, 271, 539, 565, 603, 609, 669, 687
Ichthyophthirius multifiliis 279, 289, 281
Ichthyoxenus sp. 263
Ictalurus punctatus 280, 339, 520
Ipomea aquatica 353, 354
Isistius brasiliensis 199
Isochrysis 663, 664
Isopoda 193, 194, 251, 255, 262
Kamaka sp. 194
Katsuwonus 653
Katsuwonus pelamis 163, 371, 459
Klebsiella sp. 145, 146, 236
Klebsiella pneumoniae 463, 464
Kryptopterus spp. 390
Labeo 693, 694, 695
Labeo calbasu 414
Labeo dussemerii 693, 694, 695
Labeo rohita 88, 389, 391, 414, 539, 565, 597, 603
Labeotropheus 653
Lactobacillus 464
Lactobacillus casei subsp. pseudoplantarum 443, 444
Lactobacillus plantarum 443, 444
Lagocephalidae 349, 350
Lates calcarifer 67, 243, 251, 375, 653, 664
Laurencia obtusa 290
Lecithochirium sp. 251, 252
Lecithochirium neopacificum 251, 252
Leginidium sp. 42, 212
Leiognathidae 350, 386, 418
Leiognathus sp. 459, 467
Leiognathus bindus 483, 502
Leiognathus elongatus 351
Leiognathus equulus 483, 503
Leiognathus leuciscus 502, 503
Leiognathus splendens 483, 502
Leptotheca sp. 261, 262
Lernaea 269, 270, 271, 273, 274, 275
Lernaea spp. 269, 270
Lernaea barnimiana 274, 275
Lernaea cyprinacea 263, 269, 270, 273, 274, 275
Lernaea piscinae 269, 271
Lernaea polymorpha 269, 270, 271, 273, 274, 275

Lethrinidae 478
Leucaena leucocephala 50, 122
Leuresthes tenuis 654
Limnoria sp. 630
Litopenaeus 635
Lobophyton 300
Loligo sp. 311
Lopha sp. 60
Lumbrineris meteorana 195
Lutjanidae 358, 367, 386
Lutjanus malabaricus 459
Lysiosquilla maculata 189
Macoma tokyoensis 194
Macquaria ambigua 529
Macrobrachium 6, 393, 394, 516, 647, 648
Macrobrachium dayanum 556
Macrobrachium idae 45, 46
Macrobrachium idella 647
Macrobrachium lamarrei 556, 589, 590, 647, 648
Macrobrachium lanceifrons 556
Macrobrachium malcolmsonii 647, 648
Macrobrachium nippones 397
Macrobrachium nobilii 647, 648
Macrobrachium rosenbergii 45, 46, 47, 185, 333, 334, 335, 391, 393, 394, 555, 556, 557, 615, 616, 658, 659
Macrobrachium rude 556
Magelonidae 193, 194
Mallotus 652
Matacostraca 701
Maurolicus muelleri 570
Megalaspis cordyla 372
Megalobrama amblycephala 707
Megalopidae 367
Megalops cyprinoides 368
Megelona cincta 195
Melanogrammus 654
Melanogrammus aeglefinus 653
Melanotaeniidae 367
Melicertus 635
Mene maculata 512
Mercenaria mercenaria 429
Meretrix lusoria 40
Metapenaeus sp. 581
Metapenaeus affinis 556
Metapenaeus bennettae 634
Metapenaeus endeavouri 505, 506, 507
Metapenaeus ensis 79, 505, 506, 507, 551, 629, 630
Micrococcus 235, 236, 237, 337, 438
Micrococcus sp. 331, 332, 338
Micrococcus spp. 464
Microcystis sp. 567
Micropterus dolomieu 169, 170
Micropterus salmoides 654
Microthele nobilis 478
Misgurnus anguillicaudatus 101, 105, 219
Moina 529, 530
Moina micrura 529, 530
Mollusca 193, 194, 208
Monogenea 251, 255, 265, 266
Moraxella 464
Morone 652
Morone saxatilis 652, 654
Morulius crysophegadion 390
Morus atropurpurea 157
Mougeotia sp. 567
Mugil cephalus 651
Mugil tade 80
Mugilidae 478
Mullidae 349, 350, 386
Mya arenaria 429
Mylopharyngodon aethiops 262
Mylopharyngodon piceus 157, 687
Myoxocephalus scorpioides 161, 163
Myriophyllum 689
Mystus spp. 414
Mystus nemurus 390
Mystus seenghala 415
Mytilus galloprovincialis 320
Myxobacterium 243
Myxococcus xanthus 220
Naticidae 193, 194
Navicula notha 611
Nematoda 251
Nemipteridae 349, 350
Nemipterus sp. 467
Nemipterus spp. 68, 350
Nemipterus japonicus 502
Nemipterus nematophorus 483, 502
Neomysis 114
Nephthys ciliata 194
Nephtyidae 193, 194
Nephtys 195
Nephtys ciliata 195
Nephtys dibranchis 195
Nitzschia closterium 303
Noctiluca miliaris 309
Notopterus notopterus 390
Oncaea 570, 571
Oncorhynchus 652
Oncorhynchus keta 11, 323, 324, 653
Oncorhynchus kisutch 323, 324, 669
Oncorhynchus masou 219, 323, 324
Ophicephalus striatus 242, 327
Ophieleotris aporos 368
Oplegnathus fasciatus 10, 574
Oratosquilla sp. 581
Oreochromis 201, 337, 651, 653

Oreochromis sp. 459, 460
Oreochromis aureus 37, 228, 433, 623, 624, 625, 626, 657, 676
Oreochromis esculenta 354
Oreochromis mossambicus 79, 80, 109, 117, 118, 201, 203, 255, 257, 279, 281, 337, 338, 339, 353, 354, 367, 525, 577, 578, 623, 624, 625, 626
Oreochromis niloticus 35, 37, 97, 109, 121, 141, 169, 170, 201, 203, 255, 257, 353, 354, 355, 389, 390, 391, 471, 520, 523, 524, 525, 533, 534, 535, 559, 560, 561, 599, 600, 601, 623, 624, 625, 626
Oreochromis spilurus 433
Oryzias latipes 303, 304, 687
Oscillatoria 609, 610, 611
Oscillatoria quadripunctulata 609
Osphronemus goramy 241, 390
Osteochilus 516
Osteochilus hasselti 390
Osteochilus melanopleura 390
Ostrea sp. 40
Ostreopsis 308
Ostreopsis ovata 307, 308
Ostreopsis siamensis 307, 308
Oxyeleotris lineolatus 368, 390
Oxyeleotris marmoratus 327, 390
Padina 289
Padina australis 289, 290
Padina gymnospora 290
Padina japonica 290
Padina pavonia 290
Pagrus major 113, 179, 219, 574
Palaemon affinis 630
Palaemon paucidens 397, 421
Pangasianodon gigas 390
Pangasius snitwongsei 390
Pangasius sutchi 389, 390, 683, 684, 687
Panulirus sp. 331
Panulirus spp. 381
Panulirus homarus 478, 590
Panulirus homarus homarus 381, 382, 383
Panulirus longipes 382, 383
Panulirus longipes femoristriga 381
Panulirus longipes longipes 381
Panulirus ornatus 381, 382, 383, 478
Panulirus penicillatus 381, 382, 383
Panulirus versicolor 381, 382, 383, 478
Parabramis pekinensis 668
Paracalanus 570, 571
Paralichthyidae 349, 350
Paralichthys olivaceus 219, 323, 574
Paramisgurnus dabryanus 668
Paramoeba perniciosa 331
Parapenaeopsis stylifera 418
Pelteobagrus nudiceps 398
Penaeid 581
Penaeus 506, 633, 634
Penaeus spp. 634, 635
Penaeus aztecus 633, 634
Penaeus carinatus 633
Penaeus duorarum 633, 634, 697
Penaeus esculentus 505, 506, 507, 633
Penaeus indicus 141, 331, 332, 633, 658, 659
Penaeus japonicus 595, 615, 616, 630, 633, 658
Penaeus merguiensis 331, 332, 505, 506, 507, 616, 633
Penaeus monodon 21, 22, 23, 25, 39, 40, 42, 79, 83, 84, 93, 94, 137, 207, 208, 285, 286, 331, 332, 455, 456, 459, 582, 583, 593, 594, 595, 615, 616, 617, 633, 634, 635, 637, 638, 639, 641, 642, 643, 657, 658, 659, 698, 701, 702
Penaeus notialis 697, 698
Penaeus occidentalis 658
Penaeus penicillatus 633, 634, 635
Penaeus plebejus 633
Penaeus schmitti 658, 697, 698
Penaeus semisulcatus 417, 418, 505, 506, 507, 658
Penaeus setiferus 595, 633, 634, 658, 697, 698
Penaeus stylirostris 633, 634, 698
Penaeus vannamei 633, 634, 698
Pennisetum purpureum 157
Pentaprion longimanus 350, 351, 502
Perna viridis 311, 312, 319, 320
Pharella 703
Phascolosoma 285
Photis longicaudata 194
Pinctada margaritifera 311, 478
Plecoglossus altivelis 9, 323, 687
Plesiomonas shigelloides 236
Pleuronectes platessa 651, 169
Plicatula sp. 60
Pocilopora 300
Poecilia 652
Poeciliidae 105
Polychaeta 194
Polygonum 689
Pomacentrus violascens 298
Pomadasyidae 350
Porites 300, 487, 488
Potamogeton 689
Priacanthidae 349, 350
Priacanthus tayenus 350, 351, 502
Prionospio japonicus 194
Probarbus jullieni 390
Procerovum calderoni 263
Procerovum varium 263
Prorocentrum 308
Prorocentrum concavum 307, 308
Prorocentrum lima 307, 308
Prorocentrum mexicanum 307, 308

Prorocentrum rhathymum 308
Prosorhynchus luzonicus 251
Proteus 235
Proteus sp. 236, 237
Proteus aeuriginosa 236, 237
Proteus mirabilis 236, 464
Proteus morganii 463, 464, 465
Proteus vulgaris 236, 237, 464
Protogonyaulax 307
Protogonyaulax affinis 307
Protogonyaulax catanella 307, 308
Protogonyaulax compressa 307
Protogonyaulax fratercula 307
Protogonyaulax peruviana 307
Protogonyaulax tamarensis 307, 308
Protozoa 251, 255, 256, 261, 262
Pseudodactylogyrus microchis 265
Pseudoisochrysis paradoxa 703, 705
Pseudometadena celebesensis 251, 252, 253
Pseudomonas 223, 235, 237, 243, 244, 245, 337, 338, 437, 438, 464
Pseudomonas sp. 227, 228, 235, 236, 237, 456
Pseudomonas spp. 223, 225
Pseudomonas fluorescens 237, 338
Pseudomonas putida 237
Pseudomonas/Aeromonas 244
Pseudorhombus javanicus 350, 351
Pterocladia 165
Puntius 151
Puntius spp. 414
Puntius gonionotus 71, 72, 149, 150, 390
Puntius javanicus 577, 578
Pyrodinium bahamense var. *compressa* 311, 312
Pythium 337
Pythium sp. 338
Rachycentron canadus 372
Ranina ranina 211, 212
Raphidascaris sp. 251, 252
Rhabdoviridae 324
Rhabdovirus salmonis 325
Rhinogobius brunneus 398
Rhipidocotyle sp. 252
Rhodophyceae 165
Ruditapes philippinarum 45
Ruppia sp. 137
Saccharum officinarum 157
Saccostrea sp. 60
Salmo clarki 248
Salmo gairdneri 10, 219, 280, 323, 324, 520, 653, 669
Salmo trutta 669
Salmonella 293, 437, 438, 439
Salvelinus alpinus 652, 653
Salvelinus fontinalis 298, 299
Salvinia 556, 597
Salvinia sp. 597
Sanguinicola 247, 248, 249
Sanguinicola armata 247, 248, 249
Sanguinicola davisi 248
Sanguinicola inermis 248
Sanguinicola klamathesis 248
Saprolegnia 337
Saprolegnia sp. 338
Saprolegnia diclina 339
Saprolegniaceae 339
Sarcina lutea 334
Sarcophyton 300
Sardinella 215
Sardinops sagax 652
Sargassum 290
Sargassum spp. 290
Sarocheilichthys biwaensis 398
Sarotherodon 201, 653
Sarotherodon aureus 225
Sarotherodon galilaeus 203, 623, 624, 625, 626
Sarotherodon leucostictus 354
Sarotherodon macrochir 354
Saurida sp. 467
Saurida elongata 349, 350, 351
Saurida undosquamis 483
Scapholoberis kingi 129, 133, 134
Scatophagus argus 80
Schitzothorax plagiostomus 690
Sciaenidae 386
Sciaenops ocellatus 339
Scoliodon palasorrah 459
Scomber spp. 512
Scomber australasicus 511, 512
Scomber japonicus 464, 511, 512
Scomber scombrus 228
Scomberomorus commerson 373, 473
Scombridae 386
Scorpaenichthys marmoratus 161, 163
Scylla serrata 331
Scyphidia 262
Sebastes 652
Sedentaria 194
Selaroides spp. 68
Seriola 653
Seriola quinqueradiata 219
Serranidae 386
Serrtia 145
Shigella 437, 438
Siganus 652
Siganus sp. 253
Siganus guttatus 10, 173, 174, 175, 573, 574, 653
Siliqua patula 703, 705
Sillago japonica 679
Sillago sihama 418

Silonia silondia 414
Simocephalus vetulus 133, 134
Sinonovacula constricta 703, 705
Skeletonema 26, 41, 43, 581, 582, 657
Skeletonema sp. 581, 582
Skeletonema costatum 303, 304
Solea solea 219, 651
Solen 703
Solen brevis 703, 705
Solen kempi 705
Solenidae 703, 705
Spionidae 193, 194
Spirulina sp. 42
Spirulina spp. 609
Spirulina platensis 609, 610, 611
Spondylus sp. 312
Spondylus butleri 312
Sporocytophaga sp. 220
Spratelloides gracilis 478
Squalidae 199
Squaliolus laticaudatus 199
Squilla sp. 541, 581
Staphylococcus 235, 236, 438
Staphylococcus sp. 237
Staphylococcus aureus 437, 438
Sternaspis scutata 194
Sternoptyx diaphana 570
Stizostedion vitreum 669
Stolephorus 215, 216, 217
Stolephorus spp. 215
Stolephorus buccaneeri 478
Stolephorus devisi 478
Stolephorus heterolobus 478
Stomatopoda 478
Streptococcus 438
Synodontidae 349, 350
Syringodium 507
Tachysuridae 350
Tellenidae 194
Tellinidae spp. 193
Tetraphyllidae gen. sp. 252
Tetraselmis 42, 212, 663, 664
Tetraselmis sp. 703
Tetraselmis spp. 68
Thalassia 507
Thalassia becarii 190
Thallasiosira pseudonana 705
Theora lubrica (= Th. fragilis) 194
Theraponidae 418
Thunnus albacares 371, 459
Thunnus obesus 371
Thunnus tonggol 373
Tigriopus japonicus 180
Tilapia 201, 274, 516, 653
Tilapia spp. 269
Tilapia rendalli 353, 354, 390, 525
Tilapia zillii 257, 354, 520, 525, 617, 623, 624, 626
Tor khudree 690
Tor putitora 689, 690, 691
Tor tor 414, 690
Trachinocephalus myops 349, 350, 351
Trachurus japonicus 245, 511, 512
Trachurus symmetricus 653
Trachurus trachurus 170
Transversotrema 257
Transversotrema laruei 251, 257, 261, 262
Trichoderma sp. 338
Trichodina 253, 256, 257, 262
Trichodina sp. 25, 252
Trichodina spp. 255, 256, 257
Trichodina acuta 255, 256, 257
Trichodina centrostrigata 255, 256, 257
Trichodina heterodentata 255, 256, 257
Trichodinae gen. sp. 262
Trichodinella 262
Trichodinidae gen. sp. 255, 256, 257
Trichogaster 390
Trichogaster sp. 577, 578
Trichogaster pectoralis 269, 270
Trichogaster trichopterus 257, 327
Trichopsis vittatus 327
Tridacna sp. 311
Tridacna crocea 54, 55
Tridacna derasa 53, 54, 55, 56
Tridacna gigas 53, 54, 55
Tridacna maxima 54, 55
Trochus niloticus 478
Tubifex 589, 590
Tubifex tubifex 589
Turbo marmoratus 478
Upeneus sp. 467
Upeneus moluccencis 502
Upeneus sulphureus 351
Upeneus vittatus 418
Urocleidus adspectus 266
Valamulgil seheli 478
Vallisneria 689
Veneridae 194
Veremolpa 195
Veremolpa scabra 193, 194
Verenidae 193
Vibrio 243, 244, 438, 464
Vibrio sp. 235
Vibrio spp. 332, 464
Vibrio alginolyticus 244, 245, 331, 332, 464
Vibrio angullarum 281
Vibrio campbellii 244
Vibrio cholerae 437, 438, 439, 456

Vibrio fischeri 244
Vibrio fluvialis 331, 332
Vibrio harveyi 244, 331, 332
Vibrio logei 244
Vibrio marinus 244
Vibrio parahaemolyticus 243, 244, 245, 331, 332, 437, 438
Vibrio vulnificus 244, 437
Vibrionaceae 244
Vorticella 256, 257
Vorticella sp. 255
Wallago attu 414
Xenia 300
Zoothamnium sp. 138

Author Index

Abella, T.A. 19
Abidin, A.Z. 511
Abrea, R.A. 211, 341
Acuña, R.E. 189, 211, 341
Agbayani, R.A. 523
Aguero N., M. 345
Ahmed, A.K.M.M. 153
Akimoto, Y. 193
Alava, V.R. 519
Almazan, G.J. 523
Anderson, I.G. 247
Angeles, A.F. 523
Angsinco, L.C. 189, 341, 487
Arthur, J.R. 255, 261
Arumugam, P.T. 529
Bagarinao, T. 573, 651
Baldia, J.P. 609
Baldia, S.F. 609
Bambino, C. 201
Baranda Jr., H.L. 429
Basiao, Z. 533
Baxa, D.V. 219
Becker, K. 623
Bhat, K.G. 539
Bolivar, R.B. 19
Bondad-Reantaso, M.G. 255
Borlongan, E. 581
Bulalacao, M.L. 429, 437
Caballero, R.V. 593
Camacho, A.S. 543
Caoile, S.J.S. 293, 447
Cariaga, A.M. 293, 447
Carpenter, K.E. 21, 83
Castillo, A. 671
Chan, E.H. 349
Chan, E.Y.L. 315
Chandrasoma, J. 353
Chao, Nan-Hsien 101, 105
Chao, Tien Mee 331
Chapau, M.R. 357
Chen, I-Ming 629
Chen, Jiann-Chu 657
Chen, Shyh-Jyi 101, 105
Cheng, Jin-Hua 633
Chiang, P. 25, 39
Chiba, K. 31
Chien, Yew-Hu 35
Chin, Tzong-Shean 657
Chiu, S.T. 361
Chiu, Y.N. 79, 97, 543, 547
Chong, Yen Chung 331
Chu, K.H. 551
Chua, Thia-Eng 1, 651
Chwang, N. Leh-Min 39
Coates, D. 367
Coniza, E. 141
Corre, K.C. 141, 581
Corre, V.L. 21, 121
Costa, H.H. 393, 555
Dalzell, P. 477
Dangla, E. 455
De Jesus, E.G. 637
Dela Cruz, A. 455
De Silva, C.D. 169
De Silva, S.S. 559
Destajo, W.H. 211
Devaraj, K.V. 603
Dinesh, K.R. 565
Duray, M.N. 173, 573
Duremdez-Fernandez, R. 223, 227
Estrada, M.H. 471
Fadriquela, J.F. 75
Fang, Lee-Shing 161, 629
Fang Xiuzheng 117, 125
Fang Yingxue 117, 125
Fast, A.W. 21, 83
Fernandez, S.A. 447
Ferraris, R.P. 637
Fishelson, L. 623
Flores, E. 421
Fortes, R.D. 121
Frusher, S.D. 371
Fukuyo, Y. 307
Gacutan, R.Q. 311, 429
Gallego, A.B. 173, 573
Garcia, R.P. 447
Go, You-Bong 569
Godakanda, J.C. 459
Gomez, E.D. 381
Gomez Diaz, G. 45
Grey, D.L. 375
Guerrero III, R.D. 49
Gunasekera, R.M. 559
Gunawan, G.A. 409
Guo Xianzhen 117, 125
Hara, S. 173, 573

Heslinga, G.A. 53
Hirano, R. 63, 679
Hirayama, K. 619
Hopkins, K.D. 433
Hopkins, M.L. 433
Huang Shijiao 707
Hutabarat, J. 577
Ibarra, V.M. 145
Iitaka, Y. 397, 421
Indrasena, W.M. 59
Isamu, T. 53
Ishimaru, T. 307
Janeo, R.L. 21
Jarimopas, Parnsri 109
Jiang Renliang 707
Jiffry, F. 693
Juinio, A.R. 381
Juliano, R.O. 63
Jumalon, N.A. 137
Kanazawa, A. 9
Kapur, K. 129, 133
Katase, S.A. 83
Kawai, K. 219
Keembiyahetty, C. 559
Kimura, T. 323
Kohno, H. 173, 573
Kulshreshtha, S.D. 413
Kumai, H. 103
Kungvankij, Pinij 67, 93, 581, 663
Kuo, Ching-Ming 25
Kurokura, H. 113
Kusuda, R. 219
Kuwabara, R. 193
Ladja, J.M. 637
Lai Hoi Chaw 285
Lai, Hong-Tsu 35
Lal, K.K. 129, 133
Lam, T.J. 585
Landesman, L. 585
Lee, Chin-Kai 657
Lee, Chin-Yu 35
Leong Tak Seng 243, 251
Lester, L.J. 19
Lewis, D.H. 333
Lewmanomont, Khanjanapaj 289
Liao, I-Chiu 25, 39, 101, 105, 207, 633, 641
Liew, H.C. 349
Lim, T.G. 703
Lin, C. Kwei 71
Lin, Hao-Ren 667
Lio-Po, G. 223, 227
Liu Zhiyun 117, 125
Llobrera, A.T. 333, 437
Lockwood, B.A. 345
Lopez, N.A. 75, 179
Luna, C.Z. 447
Macahilig, M.P. 547
Manalo, T.A. 523
Marian, M.P. 589, 647
Marte, C.L. 671
Mathavan, S. 589, 647
Matsuhiro, B. 165
McManus, J.W. 483
Mendoza, L.S. 443
Mendoza, N.C. 189, 211, 341, 487
Meng, Qing-Wen 401
Menon, N.R. 319
Meriwether, F.H. 675
Millamena, O.M. 593
Mines, A.N. 385
Minsalan, C.L.O. 79
Mizue, K. 199
Mohanty, S.N. 599
Moreau, J. 201
Murai, T. 641
Murugadass, S. 589, 647
Muthukumarana, G. 599
Nakamura, M. 113
Nandeesha, M.C. 539, 565, 603
Narida, M.M. 473
Natividad, J.M. 255
Ogawa, H. 289
Ogburn, D.M. 137
Ohno, A. 45
Oozeki, Y. 679
Othman, A.F. 687
Owens, J.D. 443
Palada, M.S. 19
Palpal-latoc, E.Q. 293, 447
Pandian, T.J. 589
Pang, E.K.C. 315
Pantastico, J.B. 609
Parado-Estepa, F.D. 637
Pauly, D. 15, 201, 385, 491
Pawaputanon, Oopatham 389
Perng, Chang-Yang 463
Peter, R.E. 667
Phillips, D.J.H. 315
Piedad-Pascual, F. 615
Pierce, B.E. 425
Posadas, B.C. 451
Potestas, I.O. 93, 581, 663
Prabhudeva, K.N. 319
Prager, M. 405
Premkumar, D.R.D. 589
Primavera, J.H. 593, 697
Pudadera, B.J. 93, 141
Pudadera, B.P. 581, 663

Pudadera, E. 121
Pudadera, R.A. 593
Pullin, R.S.V. 523
Quines, O.D. 145
Quinitio, G. 671
Rana, R. 87
Recksiek, C. 405
Regidor, S.E. 261
Reilly, A. 455, 473
Reyes, C.S. 341
Rubec, P.J. 297
Ruddle, K. 157
Sahaphong, Somphong 327
Saidin, T. 683, 687
Saila, S. 405
Saitanu, Kriengsag 231, 327
San Antonio, A. 533
Sanares, R.C. 83
Sastrillo, M.A.S. 543, 547
Satuito, C.G. 619
Savina, G.C. 497
Seputro, S.P. 215
Shaharom-Harrison, F. 247, 265
Shamsudin, M.N. 235
Shariff, M. 269, 273
Sharma, K.P. 87
Shetty, H.P.C. 539
Shim, K.F. 585
Shing, C.K. 551
Shreshtha, T.K. 689
Siddeek, M.S.M. 417
Silvestre, G.T. 501
Siriwardena, P.P.G.S.N. 89
Smith, I.R. 385
Smith, M.A.K. 577, 693
Sollows, J.D. 149
Sommerville, C. 269, 273, 279, 337
Soriano, M.L. 491
Staples, D.J. 505
Su, Mao-Sen 207
Subasinghe, R.P. 279, 337
Subasinghe, S. 459
Subiyanto 215
Sudhakara, N.S. 603
Suñaz, N. 437
Sun, Chao-Tung 463
Sunyasootcharee, Boonmee 327
Supriyadi, Hambali 241
Suthemechaikul, Niwat 67
Suwanrangsi, Sirilak 467
Swamy, D.N. 597
Syarani, L. 577
Sycip, M.L. 137
Tacon, A.G. 581
Takemura, A. 199
Taki, Y. 173, 573
Taleon, G.A. 141, 581
Tan, E.O. 211
Tan, M.M. 189, 341, 487
Thabrew, H. 169
Ting, T.L. 315
Tiro, L.B. 93, 663
Tokuda, H. 303
Toledo, J. 671
Tongpan, Niran 149
Torrans, E.L. 675
Traviña, R. 701
Trinidad, L.M. 471, 473
Triño, A. 701
Trio, M.G. 75
Trono, M.T.B. 523
Trujillo, L.R. 697
Tzeng, Wann Nian 511
Urzúa, C. 165
Uy, W.H. 189, 487
Vargas, R.B. 145
Varghese, T.J. 565
Vera Cruz, E.M. 185
Ver, L.M.B. 97
Vicaldo, M.V.E. 75
Vicente, H.J. 211
Villaluz, A. 227
Villegas, C.T. 701
Wang Jikun 117, 125
Wang, C.F. 703
Wang, T. 25
Wang, Tian-Sheng 401
Wanninayake, T.B. 59, 393, 555
Watson, T.C. 53
Weerakoon, D.E.M. 599
White, A.T. 497
Willoughby, N.G. 215
Woessner, J.W. 21
Wong See Yong 243, 251
Wong, T.M. 703
Wongsawang, Somatat 327
Wright, A. 477
Yamane, T. 397, 421
Yambot, A.V. 185
Yao, Chun-Tsu 35
Yoshimizu, M. 323
Zhao Weixin 707
Zhou Hongqi 707

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